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# ABNORMAL DEVELOPMENT OF TOAD OVA FERTILIZED BY SPERMATOOZOA EXPOSED TO THE ROENTGEN RAYS

BY

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WITH FIVE PLATES

## REVIEW OF THE LITERATURE

The marked alterations in structure and function produced in living tissues by the Roentgen rays and the rays of radium have excited much interest in the medical profession. Professional biologists have paid less attention to the subject, although it is one which promises to be of great importance to those engaged in experimental morphology. While probably all living tissues may be injured by sufficient exposure to these rays, there are great differences in the degree of susceptibility of different tissues and organisms. The power of assimilation of foodstuffs and their transformation into living structures complex in character seems to be that which is most altered in exposed tissues.

Bacteria are less easily affected than are most of the higher organisms. Many of those who have exposed cultures of bacteria to the Roentgen rays have failed to discover any checking of the normal multiplication of the bacteria.<sup>1</sup> Rieder,<sup>2</sup> on the other hand, found growth of bacteria inhibited by sufficient exposure to the rays. Those who have studied the action of radium on bacterial cultures have usually found the growth of the bacteria inhibited

<sup>1</sup>Park, *Medical News*, 1896; Mink, *Münchener med. Wochenschrift*, 1896; Lyon, *Lancet*, 1896; Delepine, *British Medical Journal*, 1896; Atkinson, *Nature*, lvi, p. 600, 1897 (negative results with mucedo, bacteria and oscillaria).

<sup>2</sup>Rieder, *Münchener med. Wochenschrift*, 1902.

by its rays.<sup>3</sup> Koernicke<sup>4</sup> found the development of yeast and bacteria inhibited by exposure to the rays but that the exposed cultures, if transferred to fresh unexposed gelatine, could begin to grow again.

The action of the Roentgen rays and radium on the higher plants has been recently studied by M. Koernicke,<sup>5</sup> who gives references to the previous literature on the subject. Koernicke finds that the Roentgen rays serve to check the growth of germinating seeds. Immediately after exposure there may be a slight quickening of growth. The retardation of development appears only after a latent period. If the exposure to the rays is not too intense or prolonged, the retardation of growth is merely transitory; if sufficiently intense, there is permanent inhibition of growth. Exposure of dry seeds and swollen, non-germinating seeds to the Roentgen rays had no immediate inhibitive effect on subsequent germination. Instead a slight quickening of germination was sometimes seen. Ultimately, however, the roots of the exposed seeds ceased to grow. According to Maldiney and Thouvenin<sup>6</sup> exposure to the Roentgen rays hastens germination of seeds. Lopriore<sup>7</sup> found the germination of pollen inhibited by the rays.

A pupil of H. Becquerel<sup>8</sup> found that while 24 hours' exposure of certain seeds to radium rays caused no marked diminution in power of germination, an exposure of a week or more inhibited the process. Koernicke did not inhibit growth in the seeds he studied (*Vicia faba*, *Brassica napus*, *Papaver somniferum*), but he found that exposure to radium rays caused a subsequent checking or inhibition of growth in germinating seeds and that non-germinating seeds either dry or moist, if sufficiently exposed,

<sup>3</sup>Aschkinass and Caspari: *Arch. für die Ges. Physiol.*, lxxxvi, 1901 (action attributed to  $\alpha$  and  $\beta$  rays). W. Hoffmann: *Hyg. Rundsch.*, xiii, s. 913, 1903 (bacteria killed). Dixon and Wigham: *Nature*, lxi, pp. 81, 1903 (bacteria checked in growth). Danysz: *Compt. Rend.*, cxxxvi, p. 463, 1903. H. v. Baeyer: *Zeitschrift f. allgem. Physiologie*, iv, p. 79, 1904.

<sup>4</sup>M. Koernicke: *Berichte der Deutsche Bot. Gesellschaft*, xxii, s. 163, 1904.

<sup>5</sup>M. Koernicke: *Ueber die Wirkung von Röntgen- und Radiumstrahlen auf den pflanzlichen Organismus*. *Berichte der Deutschen botanische Gesellschaft*, xxii, pp. 148-166, 1904; xxiii, pp. 404-414, 1905.

<sup>6</sup>Maldiney and Thouvenin: *Revue gen. de Bot.*, x, p. 81, 1898.

<sup>7</sup>Lopriore: *Estr. dal' Nuova Rassegna*, Catania, 1897.

<sup>8</sup>H. Becquerel: *Comptes Rendus*, t. 133, pp. 712, 1901.

germinate normally at first and then show a temporary or permanent inhibition of growth. Koernicke found in studying the tissues of exposed plants that it is the nuclei that seem especially affected by the rays. There is no visible direct injury to the cytoplasm. The effect on the nuclei is proportional to the length of exposure. The nuclei of the vegetative cells are more resistant than the pollen mother-cells. Owing to injury to the nuclei of the pollen mother-cells the pollen cells may be abnormal in appearance but this is due to the action of the injured nuclei on the cytoplasm.

In the pollen mother-cells twenty-four hours after five hours' exposure to radium rays the nuclear threads during mitosis fell into small double segments which were much smaller and more numerous than normal for *Lilium martagon* (the species studied). Division of the chromosomes took place somewhat in the normal manner but the daughter chromosomes seldom passed simultaneously toward the poles. Occasionally two or three daughter nuclei were formed on each side of the equator. If the pollen mother-cells shortly before the diakinesis of the nuclei were exposed for twenty-four hours, they showed a day later a clumping of the chromosomes at the center of the nuclear cavity. The spindle figure was strongly developed. Sometimes the spindle poles seemed split.

Exposure to radium rays up to ten hours seemed to have little effect on the daughter cells of the pollen mother-cells, but a one to three days' exposure had a marked effect. The nuclei were brought into an abnormal stage which partially resembled a resting stage. The nuclei of the tetrads arising from exposed cells were also very abnormal.

The effects of the Roentgen and radium rays on a protozoa have been studied by Schaudinn, Joseph and Prowazek, Zuelzer, and others. Schaudinn<sup>9</sup> showed that individuals of several species of protozoa may be killed by exposure to the Roentgen rays for a few hours, while others are not thus susceptible. Joseph and Prowazek<sup>10</sup> found that *Paramecia* and *Daphnia* show a negative

<sup>9</sup>Schaudinn: *Archiv. f. die gesammte Physiologie*, lxxvii, p. 29, 1899.

<sup>10</sup>Joseph and Prowazek: *Zeitschr. für allg. Physiol.*, Bd. i, 1902.

tropism toward the Roentgen rays, and that the protoplasm of *Paramecia* seems injured by the rays. I have found *paramecia* very resistant to the Roentgen rays. Twelve hours' exposure to powerful rays made no difference in the form or rate of division in *P. aurelia* or *P. candatum*. Exposure to the rays seemed not to influence conjugation. M. Zuelzer in protozoa which were exposed to radium rays under the microscope noted primarily an injury to the nuclear substance. The cytoplasm appeared affected later than the nuclei. Great variation in susceptibility was noted in different species. Zuelzer gives a brief summary of the previous literature on this subject.

Zuelzer<sup>11</sup> found insects, and Danysz<sup>12</sup> insect larvæ affected by radium rays.

The action of Roentgen and radium rays on the fertilized eggs of *Ascaris megalocephala* has been studied by Perthes.<sup>13</sup> Perthes found that there is a retardation of the cleavage of eggs exposed to the rays and that the later divisions are either inhibited or are abnormal. In the latter case the eggs give rise to either irregular masses of cells or to abnormal embryos. The effects depend largely on the degree of exposure. The nuclei of the exposed eggs are markedly affected. The chromosomes of the dividing nuclei are irregular in shape and sometimes seem to be divided abnormally into smaller parts. The spindle figures appear normal.

In fresh water Planarians Bardeen and Baetjer<sup>14</sup> showed that exposure to the Roentgen rays destroys the power of regeneration. Schaper<sup>15</sup> has shown that exposure to radium rays produces similar effects.

The action of Roentgen rays on the developing eggs of sea-urchins was found to be negative by G. Schwarz.<sup>16</sup> In the spring of 1903 the writer failed to get any positive results on exposing the eggs of sea-urchins and teleosts to the Roentgen rays, but these

<sup>11</sup>M. Zuelzer: *Archiv für Protistenkunde*, v, p. 358, 1905.

<sup>12</sup>Danysz: *Compt. Rend.*, cxxxvi, 1903.

<sup>13</sup>Perthes: *Archiv für klinische Chirurgie*, lxxi, 1903; *Deutsche med. Wochenschrift*, Nr. 17-18, 1904.

<sup>14</sup>Bardeen and Baetjer: *This journal*, i, p. 192, 1904.

<sup>15</sup>Schaper: *Anat. Anzeiger*, xxv, p. 298, 1904.

<sup>16</sup>G. Schwarz: *Wiener klin. Wochenschrift*, xvi, s. 714, 1903.



negative results he attributed to the use of an apparatus from which rays of merely moderate intensity could be obtained and which could not be used for prolonged exposures. G. Bohn<sup>17</sup> has reported the production of artificial parthenogenesis in *Strongylocentrotus lividus* by exposure to the rays of radium.

The effects of the exposure of the fertilized eggs and the larvæ of Amphibia to the Roentgen and radium rays have been studied by a number of investigators. P. K. Gilman and F. H. Baetjer<sup>18</sup> have shown that the eggs of *Amblystoma* exposed to the Roentgen rays exhibit a brief period of accelerated growth and then markedly abnormal development. If the exposure is not too severe the tadpoles may recover; if sufficiently severe they develop into monstrosities and soon die. A. Schaper<sup>19</sup> obtained somewhat similar results with frogs' eggs exposed to radium rays, although he failed to find a period of accelerated growth immediately following exposure. Schaper also found that regeneration of the tail and limbs of Triton larvæ is inhibited by exposure to radium rays. The wound heals and a mass of cells is accumulated in the region of the lost part but no specific regeneration takes place. O. Levy,<sup>20</sup> who has studied microscopically the specimens prepared by Schaper just before his untimely death, comes to the following conclusions:

- 1 In the period of cleavage of the ovum the rays may serve to check or inhibit cell division but cause no cell degeneration. Death may follow.

- 2 In the period of formation and early differentiation of the organs (generative self-assimilation) marked degenerative abnormalities appear in many of the organs, especially in the neural tube, retina and nose. The optic lens, the pigment layer of the retina, the aural vesicle, the chorda dorsalis, and the myotomes appear comparatively little affected. The heart is frequently rudimentary. The tubules of the pronephros are frequently dilated. In general, the effects are the most serious in those tissues in which growth and complex differentiation are normally most rapid.

<sup>17</sup>G. Bohn: *Comptes Rendus de l'Acad. des Sciences, Paris*, cxxxvi, pp. 1012, 1085, 1903.

<sup>18</sup>P. K. Gilman and F. H. Baetjer: *Amer. Jour. of Physiology*, x, p. 222, 1904.

<sup>19</sup>A. Schaper: *Anat. Anzeiger*, xxv, p. 298, 1904; *Deutsche med. Wochenschrift*, xxx, 1904.

<sup>20</sup>O. Levy: *Archiv f. Entwicklungsmechanik*, xxi, p. 130-152, 1906.

3 In the period of the finer differentiation of the organs (functional development of Roux) the primary effect of exposure appears to be on the blood vessels, the tissues suffering because of the effects on the blood vessels.

G. Bohn,<sup>21</sup> who studied the effects of radium rays on the eggs and larvæ of the frog and turtle, found that if growth is slow exposure to radium rays prevents the attainment of full size; if rapid and associated with tissue differentiation, radium causes degeneration of the tissues and although at first accelerating, ultimately stops development. He considers that everything leads one to think that the rays of radium affect the chromatine because it is from the activity of this substance that there results assimilation and growth.

The action of the Roentgen rays on the hen's egg has been studied by Gilman and Baetjer.<sup>22</sup> These investigators found a preliminary period of accelerated development followed by retardation of development and the production of abnormal embryos. J. Tur<sup>23</sup> studied the action of radium rays on developing hen's eggs and obtained various deformities. He found the embryonic area of the germinal disc more sensitive to the rays than the periphery and obtained some germinal discs without embryos. He found the cells of the ectoderm more easily affected than the yolk cells. G. Schwarz,<sup>24</sup> on the other hand, from his experiments on the action of radium rays on the hen's egg, concludes that the action of the rays is due to a decomposition similar to that of a dry distillation brought about in the albumenoid bodies of the cell. He explains the effect of the rays on rapidly growing tissue as due to their special power to decompose lecithin. Perthes<sup>25</sup> found that the wing of a chick exposed to radium rays was checked in its development.

The experiments with radium and Roentgen rays on plants, invertebrates and the lower vertebrates, though of great scientific

<sup>21</sup>G. Bohn: *Op. cit.*

<sup>22</sup>Gilman and Baetjer: *Op. cit.*

<sup>23</sup>J. Tur: *Comptes Rendus des Séances de la Société de Biologie*, t. lvii, 1904.

<sup>24</sup>G. Schwarz: *Archiv f. gesammte Physiologie*, C. 532, 1903.

<sup>25</sup>Perthes: *Archiv f. klin. Chirurgie*, lxxi, 1903.

value, have been comparatively few in number. The practical application of the rays in medicine has led to a much more extensive series of observations on the effects of the rays on man and mammals.

The physiological effect of the Roentgen rays first noticed was the skin burn which after an intervening latent period usually follows much exposure to the rays, and which may give rise to great thickening of the skin or to ulceration. Similar lesions were found to follow exposure to the salts of radium and like substances. Clinical experience as well as experiments on various mammals soon showed that the more deeply seated tissues, as well as the skin, are affected by the rays, but that the different tissues are variously affected. Some tissues seem to be affected directly, others seem to be affected only indirectly through alterations produced in the general metabolism or in the blood supply.

General toxic effects following the exposure to the Roentgen rays have been described in man by Seguy and Quenisset,<sup>26</sup> Walsh,<sup>27</sup> Kienbock,<sup>28</sup> Baermann and Linser,<sup>29</sup> A. S. Warthin,<sup>30</sup> D. Edsall,<sup>31</sup> and many others.<sup>32</sup> Similar effects have been described in many mammals. Tarkhanoff<sup>33</sup> experimented not only with several small mammals (mice, rabbits and guinea pigs) but also upon frogs and birds. He found that when long exposed the animals died with symptoms of paralysis. Rodet and Bertin<sup>34</sup> attributed the death of animals exposed to X-rays to a meningo-myelitis. Numerous subsequent experimenters have described toxic effects, paralytic symptoms and death in small mammals after prolonged exposure to the Roentgen and radium rays. Danysz<sup>35</sup> was one of the first to study this action of radium on small mammals. He found that a pro-

<sup>26</sup>Seguy and Quenisset: *Bulletin de l'Acad. des Sciences*, 1897.

<sup>27</sup>Walsh: *British Med. Journal*, 1897.

<sup>28</sup>Kienbock: *Wiener med. Presse*, 1901.

<sup>29</sup>Baermann and Linser: *Münchener med. Wochenschrift*, li, s. 918-994, 1904.

<sup>30</sup>A. S. Warthin, *International Clinics*, 15th series, vol. iv, p. 243, 1906.

<sup>31</sup>D. Edsall, *Journal American Medical Association*, xlvii, p. 1425, 1906.

<sup>32</sup>For a list of the literature on this subject, see Warthin, *op. cit.*

<sup>33</sup>Tarkhanoff: *Gaz. degli ospedali*, 1897. (Cited by Warthin.)

<sup>34</sup>Rodet and Bertin: *Gaz. des Hôp.*, 1898. (Cited by Warthin.)

<sup>35</sup>Danysz: *Comptes Rendus de l'Acad. des Sciences*, Paris, 1903, 1904.

longed application of the tube containing the radium salt to the head or spine of a small mammal was followed by paralysis, ataxia, convulsions and death. In the central nervous system marked hemorrhagic lesions were found after death. Similar alterations in the central nervous system have been described by Heineke,<sup>36</sup> Scholtz,<sup>37</sup> Obersteiner,<sup>38</sup> and others. Obersteiner, who paid especial attention to the lesions of the central nervous system, concludes that "the various phenomena which are observed in the exposed mice, including the death which follows sufficient exposure to the rays, in greatest part are, directly or indirectly, merely an expression of a general disturbance of the circulation and of metabolism produced by the radium rays." Obersteiner does not consider the nerve cells specifically susceptible to the rays although they are, more easily than many tissues, disturbed by alteration in the circulation or general metabolism. The general disturbances produced by the rays are indicated by the increased elimination of nitrogen discovered to take place by Baermann and Linser<sup>39</sup> after severe exposure. Lepine and Bonlud<sup>40</sup> had previously shown that alterations affecting metabolism take place in the pancreas, liver and blood after exposure to the Roentgen rays. The great susceptibility of the nervous system to the indirect, if not to the direct, action of the rays, is shown not only by the lowering of the reflexes, apathy and paralysis which precede death in animals sufficiently exposed to the Roentgen or the radium rays, but also by the injury of the retina and secondary atrophy of the optic nerve which Birch-Hirschfeld<sup>41</sup> has described. Trophic disturbances may likewise possibly be due to the injured nervous system. Obersteiner<sup>42</sup> has described a severe panophthalmitis and a gangrene of the tendons of the feet, the ears and the nose following exposure of mice to radium.

While there is doubt concerning the specific sensibility of the

<sup>36</sup>Heineke: *Münchener med. Wochenschrift*, I, s. 2090, 1903.

<sup>37</sup>Scholtz: *Deutsche med. Wochenschrift*, xxx, s. 94, 1904.

<sup>38</sup>Obersteiner: *Arbeiten aus dem Neurologischen Institute, Wien*, xii, p. 86, 1905.

<sup>39</sup>Baermann and Linser: *Op. cit.*

<sup>40</sup>Lepine and Bonlud: *Comptes Rendus de l'Acad. des Sciences, Paris*, t. xxxviii, 1904.

<sup>41</sup>Birch-Hirschfeld: *Münchener med. Wochenschrift*, 1904.

<sup>42</sup>Obersteiner: *Op. cit.*



nervous system to radium, most investigators are agreed concerning the marked action which radium and Roentgen rays have on the vascular system. Although the action of the rays on plants and ova shows conclusively that other than the vascular tissues may be directly affected by the rays, the changes in the blood vessels in young or adult mammalian tissues are among the most marked lesions found after exposure to the rays, so that the effect on other organs, as well as on the central nervous system, has been described by many as due to a secondary action on the tissues through a primary injury of the vascular system. While even the well-known lesions of the skin have been ascribed to action on the blood vessels or nerves, Oudin, Barthélmy and Darier<sup>43</sup> found in a study of Roentgen ray alopecia in guinea pigs that the layers of the epidermis were affected, the follicles and glands were atrophied but no alterations in the blood vessels and nerves of the dermis or subcutaneous were to be observed. Scholtz,<sup>44</sup> in an important contribution, concluded that both the nuclei and the cell protoplasm of the epithelial cells of the mammalian skin are injured by the rays, but that the effect on the connective tissues, elastic tissues, musculature and cartilage is slight. The skin on both sides of a rabbit's ear may be affected when it is exposed to ray on one side only. The effect on the connective tissues he thinks due to a secondary inflammatory reaction. Gassmann,<sup>45</sup> from a study of a deep Roentgen ray ulcer, concluded that the changes in the blood vessels formed the primary cause of the ulcer and its resistance to healing. Rudis-Jicinsky,<sup>46</sup> from an experimental study of X-ray burns in guinea pigs and rabbits, concluded that there is an inflammatory reaction to the X-rays followed by a development of fibrous tissue and a thickening of the walls of the blood vessels, and that degenerative changes follow the impaired blood supply. Baermann and Linser,<sup>47</sup> from a study of the action of X-rays on lupus, concluded that an endarteritis and contraction of the blood vessels with degenerative

<sup>43</sup>Oudin, Barthélmy and Darier: *Monatsch. f. prakt. Dermat.*, xxv, 1897.

<sup>44</sup>Scholtz: *Archiv f. Dermatologie u. Syphilis*, lix, pp. 87, 241, 419, 1902.

<sup>45</sup>Gassmann: *Fortschr. a. d. Geb. d. Roentgenbestr.*, 1899.

<sup>46</sup>Rudis-Jicinsky: *New York Med. Jour.*, 1902.

<sup>47</sup>Baermann and Linser: *Münchener med. Wochenschrift*, 1904.

changes in the connective tissue is the primary result of the action of the rays, the epithelium being only secondarily affected. While there is little doubt but that the deepest layer of the epidermis is affected primarily by the Roentgen and radium rays, as shown by Scholtz,<sup>48</sup> the recent work of Scholtz,<sup>49</sup> Halkin,<sup>50</sup> and others, has proved that exposure to radium rays, at least, also has a primary effect on the intima of the blood vessels. Whether the effect on the collagen of the connective tissues described by Unna,<sup>51</sup> and others, is secondary to the vascular changes or is a primary effect of the rays cannot at present be conclusively answered.

Exposure to Roentgen or radium rays has a marked effect not only on the blood vessels but also upon the blood and especially upon the blood-forming organs. Baermann and Linser<sup>52</sup> found no changes in the blood exposed to Roentgen rays. The irradiation of serum caused, however, a decrease of hæmolytic power toward blood corpuscles not exposed. Milchner and Mosse<sup>53</sup> found red blood corpuscles resistant to the Roentgen rays. London<sup>54</sup> noted a darkening of the arterial blood, and Grünbaum<sup>55</sup> hæmolysis of the red corpuscles after exposure to the radium rays.

In the spleen, lymphatic glands and bone marrow the results of irradiation are more marked than in the blood. Milchner and Mosse<sup>56</sup> found in the bone marrow of irradiated rabbits degeneration of lymphoid and myeloid leucocytes but no destruction of the red corpuscles. Heinecke<sup>57</sup> exposed rabbits, white mice, guinea pigs and dogs to the Roentgen and radium rays and found an elective action on the lymphoid elements of the spleen, lymphatic glands and bone marrow. These organs ultimately become much reduced in cell content. Warthin<sup>58</sup> began independently and

<sup>48</sup>Scholtz: *Op. cit.*

<sup>49</sup>Scholtz: *Deutsche med. Wochenschrift*, xxx, s. 94, 1904.

<sup>50</sup>Halkin: *Arch. f. Dermatologie u. Syphilis*, lxx, s. 201, 1903.

<sup>51</sup>Unna: *Deutsche med. Zeitung*, 1898.

<sup>52</sup>Baermann and Linser: *Op. cit.*

<sup>53</sup>Milchner and Mosse: *Berliner klin. Wochenschrift*, xli, s. 1267, 1904.

<sup>54</sup>London, *Berliner klin. Wochenschrift*, xl, s. 523, 1903.

<sup>55</sup>Grünbaum. (Cited by Obersteiner.)

<sup>56</sup>Milchner and Mosse: *Op. cit.*

<sup>57</sup>Heinecke: *Deutsche med. Wochenschrift*, 1903, 1904.

<sup>58</sup>Warthin: *International Clinics*, 1906.

carried farther than Heinecke an experimental study of the action of the Roentgen rays on the blood-forming organs. Warthin's paper contains an extensive list of references to the literature relating to this subject, of which use has been made in preparing the present paper. Warthin employed white rats, rabbits, Belgian hares, and guinea pigs in his experiments and subjected them to brief and to prolonged exposures. Exposures of five hours or more caused death, usually in from two to five days. In all animals exposed destructive changes were found in the lymphoid elements of the splenic pulp and follicles, marked by a degeneration of phagocytes, giant cells, and the epithelioid cells of the follicles. In all animals the effects lasted for some time and were not immediately followed by regeneration. A hæmolytic action was indicated by the great increase of blood pigment in the tissues. Warthin suggests, however, that this may have been due in part to disturbances of splenic function. A fatty degeneration was noted in the lymphoid tissues. The disintegration of lymphocytes was seen within 14 minutes after exposure and the cells continued to disintegrate for several days. The greater part of the nuclear débris was quickly removed. The Roentgen rays caused also a destruction of the lymphoid cells of the lymphatic glands. The small lymphocytes were destroyed before the other cells. Slight irradiation caused fatty degeneration; intense irradiation, nuclear degeneration. Regeneration sometimes took place after irradiation was discontinued. The effects in the bone marrow were less intense than those in the spleen and lymphatic glands. The large lymphocytes and myelocytes were chiefly attacked, the small lymphocytes not showing the marked disintegration found in the spleen. No effect on the red cells was discerned. There was an undoubted inhibition of white cell production in the marrow after irradiation.

Pusey,<sup>59</sup> Senni,<sup>60</sup> and a number of other American physicians, introduced the treatment of leucæmia by the use of the Roentgen rays. Pusey<sup>61</sup> attributes to Dr. A. J. Ochsner the first suggestion

<sup>59</sup>Pusey: Jour. Amer. Med. Association, 1902.

<sup>60</sup>Senni: New York Med. Journal, 1903.

<sup>61</sup>Pusey: Jour. Amer. Med. Association, 1905.

of this treatment. A large number of papers on the subject have been reported. A list may be found in the paper of Warthin, mentioned above. While nearly all of the leukemic cases treated have shown at first a marked improvement in the blood condition and general symptoms, there is some doubt as to the possibility of a permanent cure being brought about by irradiation with the Roentgen rays. Many of the earlier cases reported as cured have since relapsed or died. Warthin studied tissues derived from three patients who had been treated by X-ray irradiation for leukemia. In one there was a picture of an aleukemic lymphocytoma or lymphosarcoma with no lymphocytes in the vessels; in the second the tissues presented the picture of a myeloid leukemia without any changes attributable with certainty to the Roentgen ray treatment; in the third the immediate effect of the treatment was to cause in the diseased glands a fatty degeneration and necrosis of the atypical cells forming the glands. This necrosis was usually followed by an apparent sarcomatous infiltration of the surrounding tissues. In the first and third cases renal lesions suggested toxemia. The symptoms leading to death Warthin thinks are due to an intoxication resulting from the disintegration of cell proteid.

The Roentgen rays have proved of more certain value in a number of other affections, especially in acne, rosacea, nevi, lupus vulgaris, cutaneous carcinoma, and some other superficially placed diseased conditions.<sup>62</sup> In some instances the benefit seems to be due to a slight inflammatory reaction set up by the rays; in others to the alterations produced in the tissues. When the rays have direct access to the tissues, diseased cells, especially the abnormal cells which constitute carcinomata and sarcomata, may apparently be destroyed by Roentgen or radium rays before permanent injury is inflicted on the normal tissues. When filtered through normal tissues the rays seem largely to lose an elective action on the abnormal cells.<sup>63</sup>

<sup>62</sup>See Pusey, *Jour. Amer. Med. Assoc.*, 1905.

<sup>63</sup>For a list of references to the literature on the action of the Roentgen rays on tumors, see Warthin *International Clinics*, 1906. On the action of radium irradiation on tumors, see H. Rieder, *Verhandlung der Gesellschaft deutschen Naturforscher und Aerzte*, 1903, p. 278; H. Apolant, *Deutsche med. Wochenschrift*, xxx, s. 554, 1126, 1904; and Neuberg, *Zietschr. f. Krebsforschung*, ii, s. 171, 1904.



Normal or slightly diseased tissues may be rendered carcinomatous or sarcomatous. Carcinomata have resulted from the action of the Roentgen rays on the skin; and sarcomata have arisen apparently from the action of the X-rays on the lymphatic glands.<sup>64</sup>

It is well known that sterility can be brought about in man and other mammals by sufficient exposure of the testicles to the Roentgen or radium rays. Several operators have thus been rendered sterile. Of those who have studied the action of the rays on spermatogenesis mention may be made of Albers-Schönberg,<sup>65</sup> Friebe,<sup>66</sup> Scholtz,<sup>67</sup> Sedlin<sup>68</sup> and Philipp.<sup>69</sup> The exposure to the rays causes destruction of the spermatogonia and brings about aspermia.

Exposure of the ovaries to the rays causes similar disturbances. According to Halberstaeder<sup>70</sup> the ovaries are more susceptible to the rays than are the skin and testicles.

In reviewing the literature on the effects of the Roentgen and radium rays on living organisms it becomes evident that both forms of irradiation have essentially similar, if not identical, physiological action. The Roentgen rays lend themselves the more readily to exposures powerful in volume and intensity but limited in time, the radium rays are as a rule much less voluminous but can more readily be applied over considerable intervals of time. The difference in the phenomena observed after exposure to the two sources of radiant energy may perhaps be ascribed mainly to this, although it is probable that the less penetrating rays of radium modify the effects produced by the  $\gamma$ -rays. The latter are supposed to be identical with the more penetrating of the Roentgen rays. Schaper<sup>71</sup> found the frog larvæ exposed to radium emanations apparently adversely affected, yet Levy<sup>72</sup> was unable to find

<sup>64</sup>Warthin: *Op. cit.*

<sup>65</sup>Albers-Schönberg: *Münchener med. Wochenschrift*, 1903.

<sup>66</sup>Friebe: *Münchener med. Wochenschrift*, 1903.

<sup>67</sup>Scholtz: *Deutsche med. Wochenschrift*, 1904.

<sup>68</sup>Sedlin: *Fortschr. a. d. Gebiete d. Roentgenbestr.*, vii, 1904.

<sup>69</sup>Philipp: *Fortschr. a. d. Gebiete d. Roentgenbestr.*, viii, 1905.

<sup>70</sup>Halberstaeder: *Berliner klin. Wochenschrift*, 1905.

<sup>71</sup>Schaper: *Anat. Anzeiger*, xxv, p. 278, 1904.

<sup>72</sup>Levy: *Archiv für Entwicklungsmechanik*, xxi, p. 142, 1906.

in the larvæ exposed to radium emanation and preserved by Schaper, any evidences of tissue degeneration such as follows direct irradiation.

The Roentgen rays and the rays emitted by radio-active substances are known to cause ionization of gases, to affect photographic plates, phosphorescent substances and glass. It seems evident that they also modify the chemical nature of living bodies. It is certain that in some instances, at least, as proved by Perthes<sup>73</sup> Thies,<sup>74</sup> Koernicke,<sup>75</sup> and others, the cell nuclei are primarily affected by the rays. An injury to nuclei of cells sufficient to destroy the normal influence over metabolism would suffice to account for all phenomena which have been observed. The special destructive influence on cells undergoing rapid assimilation, multiplication and differentiation may be accounted for by the very important rôle played by the nuclei in these processes and an apparently greater susceptibility of the nuclei at such periods. The recent important and suggestive paper of F. R. Lillie,<sup>76</sup> on the elementary phenomena of embryonic development in *Chætopterus*, shows what an active part is performed by the nuclei of cells during the early stages of embryonic development in furnishing substances to the cytoplasm and in turn assimilating substances from the cytoplasm. While irradiation does not as a rule directly stop the phenomena of mitosis it evidently severely alters the productive activities of the nuclei of the cells and thus affects metabolism. Irradiation may also possibly directly affect the cytoplasm and intercellular substances.

Thies<sup>77</sup> from a careful study of the action of radium rays on the different mammalian tissues and organs comes to the conclusion that all tissues suffer, although the elastic tissues are relatively resistant. The adenoid tissues are the most susceptible. Other very susceptible tissues are the epidermis, the intima of the blood vessels, the parenchyma of the sex glands, voluntary muscle, white

<sup>73</sup>Perthes: *Archiv f. klin. Chirurgie*, lxxi, 1903; *Deutsche med. Wochenschrift*, 1904.

<sup>74</sup>Thies: *Wirkung der Radiumstrahlen auf verschiedene Gewebe und Organe. Mitteil. aus den Grenzgebieten d. Medizin und Chirurgie*, xiv, 1905.

<sup>75</sup>Koernicke: *Berichte der deut. bot. Gesellschaft*, 1904-05.

<sup>76</sup>Lillie: *Jour. of Experimental Zoölogy*, iii, pp. 154-263, 1906.

<sup>77</sup>Thies: *Op. cit.*

fibrous tissues and cartilage. Neither Scholtz<sup>78</sup> nor Danysz<sup>79</sup> place the connective tissues or voluntary muscle among the tissues especially susceptible to irradiation.

The physiological chemical effects of irradiation are not yet clear. While some authors attribute much to the decomposition of lecithin, others deny that irradiation can decompose this substance. There is some evidence that the action of ferments is influenced by irradiation.<sup>80</sup>

#### EXPERIMENTS

The following experiments were designed primarily to test whether nuclear alteration produced by exposure to the Roentgen rays would alone suffice to cause the tissue alterations characteristic of this exposure. For this purpose I exposed spermatozoa to the X-rays and then fertilized eggs with these spermatozoa. Since the chief portion of the spermatozoön is the nucleus and since the mass of spermatozoön is insignificant compared to that of the egg it seems fair to conclude that if the exposure of the spermatozoön to the rays influences the development of the ovum the action of the rays must be on those unknown substances in the nucleus, or the protoplasm most intimately associated with the nucleus, which control the morphogenetic activities of the cell.

The toad was selected because it is comparatively easy to get both males and females at the height of sexual maturity and the spermatozoa will live for several hours in water after removal from the body. My procedure was to collect several pairs of toads, separate the males from the females and wash the latter for some time in running water. From the testicles and Wolfian ducts of two of the males a thick suspension of spermatozoa was obtained. This was slightly diluted and divided into two portions, one of

<sup>78</sup>Scholtz: *Archiv f. Dermatologie u. Syphilis*, lix, 1902; *Deutsche med. Wochenschrift*, **xxx**, 1904.

<sup>79</sup>Danysz: *Comptes Rendus de l'Acad. des Sciences*, Paris, 1903, 1904.

<sup>80</sup>Schwarz: *Archiv f. die gesammte Physiologie*, c. s. 532, 1903; Baermann and Linser: *Münchener med. Wochenschr.*, li, 1904; Danysz J.: *Comptes Rendus de l'Acad. des Sciences*, Paris, 1903, 1904; Henri and Mayer: *Comptes Rendus*, 1904; Lepine and Bonlud: *Comptes Rendus*, 1904; Neuberg: *Zeitschr. f. Krebsforschung*, ii, s. 171, 1904; Harry: *Journal of Physiol.*, **xxix**, 1904; E. Benjamin and A. V. Reuss: *Münch. med. Wochenschr.*, liii, 1906.

which was kept for control, the other was exposed for from one-half to two hours to powerful X-rays from a fairly hard tube. At the end of the exposure I opened several females to obtain if possible eggs immediately ready for laying. Several short pieces of strings of eggs were placed in the control dish and others in the dish which had been exposed to the rays. After fifteen to twenty minutes these strings were removed and placed in large dishes of water. The development of the control and the experiment ova was then watched from day to day until it seemed likely that the ova fertilized by the exposed sperm could no longer survive. Specimens of the control and experiment embryos were then preserved for microscopic study.

Several of the experiments proved of negative value because not even the spermatozoa of the control dish proved capable of fertilizing the eggs, owing either to too great a lapse of time between the removal of the sperm from the males or to a lack of sufficiently ripe ova. The season was so short that only a few successful experiments could be carried out but these were convincingly positive. All eggs fertilized by the control spermatozoa developed normally. One of these was finally attacked by some parasitic organisms, but even in this the tissues and organs showed microscopically perfectly normal relations. All eggs fertilized by the exposed spermatozoa, over fifty in number, developed abnormally, with a single exception. This ovum developed into an apparently normal tadpole but this tadpole died before any of its fellow control tadpoles. It died at a time when it could not be immediately preserved for microscopic examination.

### *Outline of Experiments*

I, May 4. Owing, probably, to too great dilution of sperm and too great a length of time between removal from the body and the attempt at fertilization of the ova, neither the irradiated or the exposed sperm fertilized any ova.

II, May 5. Sperm exposed for two hours to X-rays about four inches from the tube. A large number of the spermatozoa were active at the end of this period. After fertilization the control and



experiment ova were divided into two lots, one of which was kept in lake water and one in the harder city water.

May 6. Between 50 and 75 per cent of the eggs in each of the four lots were in the blastula stage.

May 7. All fertilized eggs were in the gastrula stage.

May 8. All fertilized eggs were advanced to the stage in which the yolk plug appears small.

May 9-22. The eggs in the city water during this period developed much more slowly than the lake water eggs. The control eggs in the city water were apparently nearly all fertilized and developed apparently normally though slowly up to May 12 when, owing to the cover being left off the shallow dish in which they were contained, and consequent evaporation of water during the absence of the writer from the city, the whole lot was destroyed. Of the experiment ova kept in city water about 50 per cent developed. On May 11 seventeen unfertilized and partially developed eggs were removed, leaving eleven nearly normal larvæ with very short caudal processes. On May 13 only six of these were still alive. These six were then transferred to lake water. Two could move about by movements of the body and tail. The other four showed little power of motion. These four larvæ and one of the two more motile ones did not grow much more in size but became quite abnormal in external form similar to those pictured in Plates II to V. One of the two more motile larvæ developed into a tadpole fairly normal in external form, although it died before any of the controls which were kept in the lake water.

Of the control ova in the lake water four eggs were unfertilized and nine developed into perfectly normal tadpoles which were kept alive for several weeks. Of the experiment ova on May 11 eight unfertilized or undeveloped eggs were removed, leaving seventeen larvæ with slightly developed heads and very short caudal processes. On May 13 the control larvæ could swim for short distances. None of the experiment larvæ could do so. Externally the latter had begun to exhibit various abnormalities which became more marked from this period on. About a dozen of these larvæ were preserved on May 14. The others all died a few days later, *i. e.*, in from ten to fourteen days after the

exposure of the sperm. The external form of some of the larvæ preserved is shown on Plates II to V.

III, May 14. Unsuccessful because ova proved unripe. Between May 5 and May 13, owing to a spell of cool weather, no toads could be obtained.

IV, May 14. The day was very warm. At the end of the hour and half during which spermatozoa were exposed to the rays both experiment and control spermatozoa were non-motile and proved incapable of fertilizing any ova.

V, May 15. Sperm exposed an hour and a half. A considerable proportion of the control ova were fertilized. Only a few of the experiment ova were fertilized. Of these only one developed past the early cleavage stages. On May 22, at three o'clock, p. m., this larva, which was small, unsymmetrical and ill-developed, appeared about to die. It was, therefore, killed and preserved. Microscopically it presented the features characteristic of the tadpoles developed from ova fertilized by irradiated sperm.

VI, May 16. Sperm exposed one and a quarter hour. At the end of this period neither irradiated nor control spermatozoa proved capable of motion or of fertilizing. The day was an exceedingly warm one.

VII, May 16. Sperm exposed one-half hour to the rays. Of the control ova only about 20 per cent were fertilized. These developed normally. Of the experiment ova only three or four per cent were fertilized. None of these developed past the gastrula stage.

### *Structure of the Larvæ*

At the time of or soon after the hatching of the larvæ abnormal structural differentiation began to make an externally visible distinction between those experiment larvæ which developed best and the control larvæ. In the experiment larvæ the tails all showed more or less abnormality of form and the larvæ with a few exceptions proved incapable of swimming, although some were capable of irregular movements of the body and tail. In length the experiment larvæ did not grow much after this period. In width and thickness the growth was greater but was in large part

due to abnormal distension of various regions with fluid. The external form meanwhile became most irregular. This irregularity of external form is more easily illustrated than described. See outlines on Plates II to V. At the period when the sketches were made of the larvæ here illustrated the control larvæ had the external form shown in the outline on Plate I. The rudiments of the hind legs were beginning to be externally visible.

The organs of the experiment larvæ show the same irregularity of form as that shown by the body as a whole. Wax model reconstructions of the organs of each individual larva which has been preserved and of a series of stages of the normal larvæ of the toad would be necessary for a thorough study of the abnormalities which exist in the experiment larvæ. It has not seemed worth while to make so elaborate a study because each experiment larva is affected in such an individual way that it seems improbable that broader generalizations could be drawn from such a study than from the simple inspection of the serial sections of the larvæ.

#### Control Larva of the Size of the Experiment Larvæ

The control larvæ at the period when the experiment larvæ began to show marked abnormalities in external form were about at the stage described by Marshall in his *Vertebrate Embryology* as characteristic of the tadpole of the frog shortly after hatching. The organs may be briefly described as follows:

*Nervous System*—The central nervous system is slightly more advanced than at the stage of development described by Marshall in the newly hatched tadpole of the frog. The forebrain extends anteriorly between the olfactory pits. The end brain is beginning to be divided anterior to the olfactory pit into two hemispheres. In the groove between these is a vascular plexus. The pineal body extends forward above the undivided portion of the forebrain. It is composed of cords of cells interlaced with capillaries. The optic stalks are patent near the neural canal but toward the optic cups are narrow and are apparently partly filled by newly formed fibers. The infundibulum is large, its lateral walls are thick. The roof of the midbrain has begun to thicken. The floor of the fourth ventricle on each side is very thick. The

spinal cord may be followed well into the tail. It gradually becomes narrow and more rounded as one passes posteriorly. The neural canal is relatively large.

*Organs of Special Sense*—The nasal pit is connected with the pharynx by a solid column of cells. The thickened lateral wall of the brain projects against the olfactory pit and the olfactory nerve is in process of formation. The lens of each eye is in contact with the ectoderm. Although differentiation of the lens is well under way it still contains a vesicular cavity. The sensory layer of the retina is thick and closely applied to the pigment layer. As mentioned above, the optic stalk near the optic cup is small and seems to contain nerve fibers.

Each auditory vesicle is a simple closed sac with a short blind diverticulum. The auditory ganglion consists of a mass of cells situated between the anterior portion of the auditory sac and the medulla.

*Peripheral Nerves*—The trigeminal ganglion is large. It is not in connection with the ectoderm. The ganglion of the facial nerve cannot be sharply distinguished from that of the auditory nerve. The glossopharyngeal and the vagus ganglia are well developed but it is difficult to trace the chief branches of these nerves for any considerable distance. The spinal ganglia consist of well marked groups of cells lying ventro-lateral to the spinal cord. The nerve fibers of the spinal nerves are beginning to form in the proximal spinal segments.

*Alimentary Canal*—The shallow stomodæum opens into the pharynx. The lips and jaws of the stomodæum are beginning to grow forward.

The broad pharynx communicates by gill slits with the exterior. The external gills are fairly well developed. The opercular fold is beginning to be formed.

The anterior part of the lumen of the œsophagus is blocked up by epithelial cells. The pulmonary diverticula are small lateral out-growths which extend but a short distance posteriorly from the œsophagus.

Passing back from the blocked region of the œsophagus the gut makes a curve to the left about the liver and pancreas, and then

terminates in a mass of cells filled with yolk and presenting no well marked lumen. Toward the posterior end of the trunk a lumen appears in the dorsal region of this mass. This lumen may be followed to the anus.

The sinusoidal circulation of the liver is well established. The bile duct is of some length and opens into the intestine slightly to the left of the mid-line of the body. The anlage of the gall bladder may be seen below the bile duct.

The anlage of the pancreas is marked by a deeply staining mass of cells posterior to the anlage of the liver on the left side of the body. The ducts at this stage are not clearly marked in this embryo.

*Heart and Blood Vessels*—The heart is an S-shaped tube, the lumen of which is filled with blood. There are no well-marked trabeculæ in the ventricle. The main arteries and veins are filled with blood and may be readily followed. In general they seem to correspond with the diagrams of the blood vessels for the recently hatched tadpole given by Marshall (*Embryology*, p.170, Figs. 77 and 78).

*Reproductive Organs*—The coiled tubules of the head kidney are surrounded by a vascular plexus. Distally the two Wolfian ducts have a common opening into the cloaca. They are patent throughout.

*Skeleton*—The notochord and a loose mesenchyme formed of anastomosing cells constitute the skeletal tissues of this embryo.

*Muscles*—In the region of the head the muscles anlagen are marked by dense masses of tissue, in some of which specific differentiation has begun. In the region of the spinal cord the myotomes form a well differentiated segmental musculature on each side of the chorda dorsalis, the spinal cord and the spinal ganglia.

#### Control Larva of the Age of the Experiment Larvæ

The control larvæ, Plate I, at the period when most of the experiment larvæ were preserved for examination were about at the stage of development described by Marshall for the 12 mm. tadpole of the frog. The organs may be briefly described as follows:

*Central Nervous System*—The olfactory lobes extend between the nasal organs. They are beginning to be fused in the region shown in section *a*. Anterior to this they are more clearly separated and posterior to it the lobe on each side is continued into a well marked cerebral hemisphere. Section *b* passes through the brain slightly anterior to the junction of the two lateral with the third ventricle. The anterior end of the choroid plexus is shown between the two lateral ventricles and above this the posterior portion of the pineal body. The latter extends forward for a considerable distance beneath the ectoderm. At this period it contains a small vesicular cavity. The lateral walls of the third ventricle are thick (section *c*). The optic stalk has been converted into an optic nerve but has a short patent lumen near the third ventricle. This does not appear at the level of section *c*. The infundibulum is large. The opening into it from the third ventricle is small, but beyond here it rapidly expands (section *d*). The optic lobes project dorsally on each side of the midbrain (section *d*). The cerebellum is marked by a slight thickening of the anterior margin of the roof of the fourth ventricle. The ventro-lateral walls of the medulla are very thick (section *e*). The folds of the choroid plexus are beginning to appear in its thin roof. The spinal cord is well developed and extends far back in the tail (sections *f*, *g* and *h*). The Randschleier, in the region of the trunk, is as thick as the layer of cells surrounding the central canal.

*Organs of Special Sense*—Large nasal fossæ extend from the exterior to the pharynx. The epithelium of the medial wall of each fossa is very thick (section *a*). Cæcal outgrowths are taking place from the posterior dorsal portion of each fossa. Bundles of nerve fibers may be traced from the lateral side of each olfactory lobe to the thickened epithelium of the corresponding nasal fossa.

The eyes (section *c*) are well developed. The several layers of the sensory part of the retina may be distinguished. A thin sclerotic coat is present. The well formed lens is separated by a distinct interval from the ectoderm.

The auditory vesicles (section *e*) are being divided by the ingrowth of septa into the various portions characteristic of the adult ear. The auditory nerve is undergoing rapid development.



*Peripheral Nerves*—The ganglion of the trigeminal nerve is highly developed. In section *d* a portion of it may be seen on each side of the midbrain. The main branches of the nerve may be followed for some distance from the ganglion. The ganglion of the facial nerve is still so close to that of the auditory nerve that no sharp line of division can be seen in the sections. The glosso-pharyngeal and vagus ganglia are likewise still difficult to differentiate from one another in the sections. The spinal ganglia are well differentiated. The motor and sensory roots and the main trunks of the spinal nerves may be distinguished without difficulty.

*Alimentary Canal*—The lips and beak are highly developed. Section *a* shows on each side of the oral opening a section of the lower jaw tipped by an epithelial tooth. The operculum is attached to the ventral and right sides of the body posterior to the gills. Section *e* shows the opercular cavity near the anterior end of the heart. The internal gills are of considerable size. Only traces of the external gills remain. Over the region of the heart the ventral wall of the pharynx gives rise to a medial projection which extends in a posterior direction in the pharynx. Near the tip of this the trachea arises as a solid column of cells from the ventral wall of the alimentary canal near the junction of the pharynx and œsophagus. More distally two tubular pulmonary processes extend back one on each side from the trachea along the dorsal wall of the body cavity. These do not reach so far as the anlage of the pancreas. The œsophagus narrows rapidly posterior to where the trachea is given off. It diverges at this period toward the right side of the body (see section *f*, immediately in front of and at the left of the chorda dorsalis). It joins the stomach in front of the pancreas. In section *f*, on the left side of the figure (right side of the body), the stomach is shown cut through in two places and between these two sections of the stomach the anterior portion of the pancreas shows on each side. The two regions of the stomach here shown are joined together anterior to this section. The ventral portion is joined with the œsophagus posterior to the level of the section. The dorsal portion of the stomach shown in this section passes posteriorly into a much coiled intestine. Part of the coils of the intestine pass ventral to the heart, anterior to the

liver. The liver and gall bladder are highly differentiated. The posterior end of the liver and the bile duct are shown between the œsophagus and stomach in section *f*. The gall bladder lies dorsal to the liver somewhat anterior to the level of this section. The entrance of the bile and pancreatic ducts into the gut takes place considerably posterior to this level. The anus in this embryo passes out between the anlagen of the posterior limbs (section *g*).

*Heart and Blood Vessels*—The heart is much larger than that in the control embryo previously described. The ventricle is crossed by large trabeculæ (section *e*). The auricle is beginning to be divided into right and left halves. The blood vessels are dilated with blood. The vessels seem to correspond with those described for the 12 mm. tadpole by Marshall (Embryology, Fig. 76, p. 166).

*Genito-Urinary Organs*—The tubules of the head kidney are greatly coiled. The tubules of the mesonephros are differentiated near the cloaca. More anteriorly the anlagen of these tubules consist of dense masses of cells. The genital folds are not prominent.

*Skeleton*—The differentiation of the vertebræ has scarcely begun in this embryo. The chief cartilages of the skull have appeared (see Marshall, Embryology, p. 262, Figs. 90, 91 and 92). A moderate amount of mesenchyme surrounds the different organs.

*Muscles*—The chief muscles of the head are clearly differentiated. In the trunk the myotomes are larger than at the preceding stage and the muscle cells are more highly developed.

The abnormalities exhibited by the experiment larvæ may be illustrated by a few typical examples.

#### Larvæ No. 1, Experiment II, Plate II

*External Form*—This embryo exhibits a marked dorsal flexion of the body near the middle of the trunk, so that the long axis of the tail is at right angles to that of the anterior portion of the trunk and the head. The bend seems due largely to an abnormal dilatation of the hind-gut. The head is quite irregular in shape, the deformity of outline being due to great abnormality in the

internal structures. Posterior to the mouth the sucker is well developed on each side (section *b*).

*Central Nervous System*—The brain ends anteriorly in a hollow rounded protuberance from which a vesicular ventricle projects on each side (section *a*). The brain does not extend anteriorly to the olfactory pits. The walls of the telencephalon and the hemispheres are irregular in thickness and the cells and fibers are very abnormally disposed. Many pigmented and apparently degenerate cells lie in the walls of the third ventricle and free in the ventricle. The pineal gland lies between the roof of the third ventricle and the ectoderm in a region posterior to section *a*. It has a thin wall and a hollow central cavity containing scattered cells which show evidences of degeneration. As one passes back the neural canal becomes much dilated. In the region of the optic stalk it is thin-walled (section *b*). The hypophysis is somewhat dilated but is relatively normal in structure (section *c*). The roof of the midbrain is rather thin. In the region of the ears the left side of the brain is much less developed than the right side (section *d*) and posterior to the ears for a considerable distance the left side of the neural tube consists of hardly more than an irregular membranous wall. Near the middle of the trunk the spinal cord is more symmetrical, but posteriorly it is once more undeveloped on the left side (section *g*).

*Organs of Special Sense*—The nasal pits on each side are connected with the pharynx by columns of cells (section *a*) which show near the pharynx an imperfect lumen. The eyes are irregular in form (section *b*). A cornea has been differentiated. The sensory portion of the retina is poorly developed. The lumen of the optic stalk is dilated and extends into a space between the two layers of the retina. There are no nerve fibers (section *b*). The pigment cells of the pigment layer are irregularly disposed and project out into the neighboring tissues. The auditory sacculus consists of a rounded pouch with a dorsal diverticulum (see *d*). The auditory ganglion is clearly marked but no nerve fibers can be distinguished.

*Peripheral Nerves*—The cells of the ganglion of the trigeminal nerve are large and well differentiated. Nerve fibers cannot

readily be followed. The ganglia of the ninth and tenth nerves are present on the right side only and are rudimentary. Rudimentary spinal ganglia are present except where the left side of the spinal cord is defective. No nerve fibers can be distinguished.

*Alimentary Canal*—Lips and beak are differentiated, though abnormal in form. Section *a* passes through the lower jaw and lower lip. The oral opening into the pharynx is large. The internal gills are irregularly developed and contain no blood vessels. The œsophagus is patent and extends to the left into a thick-walled stomach. In the latter part of its course the lumen is divided into three flues, all of which open into the stomach (sections *e* and *f*). The tracheo-pulmonary process is short, does not branch and has a slight lumen. The liver is developed posterior to the region of the heart and ventral to the stomach (section *f*). A few blood corpuscles can be seen in the much dilated capillary spaces of the liver. The pancreas is developed on the left side of the body posterior to the stomach. It consists of columns of cells. From the stomach the gut passes into an irregular group of coiled intestines, situated well on the left side of the body in the anterior region of the body cavity (section *e*), but in the middle more posteriorly (sections *f* and *g*). The hind gut is greatly distended. The anus seems to be occluded. The epithelium over the anus extends outward with irregular branching processes.

*Circulatory System*—The heart is occluded and contains no blood. The only blood vessels clearly distinguished are a portion of the dorsal aorta, the mesenteric artery, and a few venous sinuses in the liver. In these vessels a few blood corpuscles are scattered about.

*Genito-Urinary System*—The tubules of the pronephros are much dilated. There are a few glomerular tufts but these contain no blood vessels. The Wolfian tubules are abnormally dilated (sections *e*, *f* and *g*).

*Skeleton and Connective Tissue*—The connective tissue is in most regions excessive in amount. Pigment cells are irregularly scattered about. Most of the cartilages of the head seem to be present but the tissue is not thoroughly differentiated and there are some abnormalities of form. The auditory capsule is not

differentiated. The chorda dorsalis is fairly regular in form except at the anterior end, where some of the cells seem completely to have disappeared, and in the region of the medulla, where it is asymmetrical in places.

*Musculature*—A number of the muscles of the head are fairly well developed. The individual muscle cells are in some instances highly differentiated. The myotomes are fairly normal except next the undeveloped region of the spinal cord on the left side.

*Skin*—In several places there are villus-like outgrowths of epithelium.

### Larva No. 2, Experiments II, Plate III

*External Form*—The caudal extremity of the embryo bends sharply in a dorsal direction. The body cavity is enormously distended, although the alimentary canal is but slightly developed (see sections *c* to *i*). In these sections the ventral wall of the body cavity has collapsed, owing to the action of the fixing fluids. The head is exceedingly irregular in shape, owing to the imperfect development of the organs of special sense and the abnormal accumulation of a loose mesenchyme. The mouth opens on the back of the head anterior to the anterior nares. Apparently there is no sucker. Along the dorsal margin of the posterior end of the body and the tail, folds of tissue project (sections *b* and *i*).

*Central Nervous System*—The ventral end of the central nervous system consists of a thin-walled dilated sac which does not extend as far forward as the nasal fossæ. Sections *a*, *b* and *c* show no trace of it. Sections *d*, *e* and *f* show the abnormal condition of the walls of the neural tube. There is no fibrillar framework (Randschleier). The cells are irregularly placed; many of them show evidences of degeneration and not a few lie free in the neural canal. The pineal gland is a small vesicular pouch, the walls of which are composed of cells which exhibit degeneration. The infundibulum consists of a thin-walled projection from the ventral portion of the neural canal. Section *d* shows the entrance into it. The spinal cord consists of a round thin-walled tube in which no specific differentiation has taken place.

*Organs of Special Sense*—The nasal fossæ are patent but irregular in form (sections *b* and *c*). No olfactory nerve can be distinguished. The eyes are very rudimentary (section *d*). The lens consists of a small round clump of cells. The optic stalk is patent and the pigment and sensory layers of the retina are separated by a space connected with the lumen of the eye stalk. The sensory layer consists of a thin membrane of partly degenerate cells. The cells of the pigment layer are irregular in outline. The auditory vesicles are small round sacs from each of which a short dorsal diverticulum extends. The auditory nerve is not distinguishable.

*Peripheral Nerves*—The ganglion of the trigeminal nerve is partly developed. Those of the ninth and tenth nerves are not distinct. No spinal ganglia can be distinguished with the exception of one small group of cells in the mid-thoracic region.

*Alimentary Canal*—An irregularly shaped mouth extends from the dorsal surface of the head to the pharynx. Its margins are surrounded by folds of tissue (section *a*). The pharynx is dilated near the mouth (section *b*). Posteriorly it is flattened from front to back (sections *c* and *d*). The gill clefts are patent. From the septa which partially close them irregular outgrowths arise (section *d*). The œsophagus passes posteriorly from the right side of the pharynx (section *e*). At the left of the origin of the œsophagus there is a mass of cells, flat in cross section, which is continued for some distance along the dorsal wall of the body cavity (section *f*). This represents the tracheo-pulmonary anlage. The œsophagus passes into the stomach at the left of the liver (section *e*). The gut curves in front of the pancreas (section *f*), whence it is continued into a straight gut which about the middle of the trunk exhibits a distinct lumen that is continued with some interruptions to the rectum (sections *b* and *i*) and through the anus to the exterior. In addition to the main lumen several irregular tubular spaces occur in the mass of cells which compose the gut. The greatly dilated body cavity extends far forward beneath the pharynx. The liver is partially differentiated, extends forward beneath the pharynx and posterior to the rudiment of the heart has a few blood sinuses containing blood corpuscles. The pancreas is partially differentiated posterior to the liver and dorsal to the stomach. In

section *f* it may be seen between the gut and the tracheo-pulmonary process.

*Heart and Blood Vessels*—The heart apparently consists of a thin-walled tube which has been ruptured. The only blood vessels which can be made out in the embryo are irregular sinuses containing a small number of blood corpuscles.

*Genito-Urinary Organs*—The pronephric tubules are rudimentary and are irregularly drawn out in the distended wall of the body cavity (section *f*, on the right side of the section). The Wolfian ducts cannot be followed uninterruptedly to the cloaca. They lie far out in the body wall on each side. In places they are dilated, in places apparently missing.

*Skeleton and Connective Tissues*—The anterior end of the chorda is small and defective (section *f*). In the spinal region it is relatively normal (sections *b* and *i*). There is an excessive amount of loose mesenchyme throughout the body, especially in the region of the head. A few of the cartilages of the head are developed, though apparently not perfectly normal in form.

*Musculature*—In the head a few groups of partly differentiated muscle cells indicate muscles. The outlines of the trunk myotomes are not distinct. The muscle cells are more scattered than normal, owing apparently to their being forced apart by the invasion of fluids and mesenchyme.

*Skin*—There are numerous places in which the epithelium gives rise to finger-like projections. The most marked of these is the dorsal margin of the trunk and the tail.

### Larva No. 3, Experiment II

*External Form*—The posterior half of the body, including the tail, bends sharply in a dorsal direction. This is due to a great dilation of the hind gut. The head is rounded and swollen and it is difficult to make out clearly the specific characters of the tadpole head. The sucker is rather flat.

*Central Nervous System*—The central nervous system ends anteriorly in two distinctly separated olfactory lobes into which the lateral ventricles extend but a short distance. The walls are abnormally differentiated and contain both cells and fibrous tissue,



(Randschleier). There is no pineal gland but the roof of the third ventricle projects inward in such a way as to suggest an inverted pineal gland. It is different from the inversion which accompanies the choroid plexus. The ventral wall of the brain is thick where the optic stalks arise. The infundibulum is large and contains many desquamated cells. The roof of the midbrain is thin, although there are some evidences of the rudiments of the optic lobes. The medulla is relatively normal. A considerable mass of tissue, resembling yolk cells, lies free in the fourth ventricle. The proximal part of the spinal cord is fairly well developed. Posteriorly in places the spinal cord is irregular in form.

*Organs of Special Sense and Peripheral Nerves*—On the right side a nasal fossa extends from the nasal pit to the pharynx. On the left side a column of cells with an imperfect lumen does not quite reach the pharynx. Near the pharynx it gives off a lateral process. The optic stalk is patent on each side and the cavity extends between the pigment and sensory layers of the retina. The sensory layer is not specially differentiated. The cornea is closely applied to the front of the sensory layer and lies at some distance from the ectoderm. The auditory vesicles are somewhat simple sacs with dorsal diverticula. The auditory nerve may be followed into the brain. The sensory ganglia are for the most part fairly well developed. The peripheral nerves cannot be followed.

*Alimentary Tract*—There is a free opening into the pharynx. This is surrounded by imperfectly developed lips and jaws. On the lips and the jaws are numerous imperfect teeth. Internal gills are fairly well developed on the left side but on the right side form a dense mass fused with the operculum. The opercular cavity does not extend ventral to the pericardium. The œsophagus is nearly occluded by epithelial cells. From it a solid cord of cells passes posteriorly and represents the pulmonary anlage. As the œsophagus is continued into the stomach the alimentary canal swings to the left of the liver and pancreas. The gut then passes to the right across the front of the body, then anteriorly and finally curves back and passes into a portion of the gut which is much distended and extends posteriorly for some distance, then takes

several coils forward and finally passes back into the rectum. This is greatly dilated and partly coiled. The anus is small and apparently partly stopped up by mucus. The ventral wall of the body cavity is strikingly thick, not only in front of the heart but also over the entire abdomen to the anus.

*Heart and Blood Vessels*—The heart is thin-walled but fairly well formed. There are some trabeculæ in the ventricle. The pericardial cavity is small, owing to the great amount of mesenchyme in the body wall. Some, at least, of the chief blood vessels are present and contain a considerable amount of blood.

*Genito-Urinary Organs*—The tubules of the head kidney are irregular in form and are much dilated in places. The Wolfian ducts may be followed to the cloaca. Near the cloaca the anlagen of the tubules of the mesonephros may be seen.

*Skeleton and Connective Tissue*—The chorda is relatively normal. An excessive amount of mesenchyme is present, especially in the region of the head. In the head most of the cartilages are fairly well developed.

*Musculature*—In the head the muscles are fairly well differentiated. The myotomes are fairly normal although the cells are somewhat more scattered than usual.

*Skin*—In places irregular finger-like processes of the epithelium project from the body.

#### Larva No. 4, Experiment II, Plate IV—A

*External Form*—The tail is a mere rudiment. The ventral part of the body is much swollen, owing to distention of the body cavity. The head is small and deformed. The sucker is rather flat (section *a*).

*Central Nervous System*—The neural tube ends anteriorly in two thick walled olfactory lobes. The right one is shown on the left side of section *a*. The cells in the walls are irregularly placed and many exhibit signs of degeneration. The Randschleier is not normally disposed. There is a small pineal body with a narrow lumen and thick walls composed of cells not specifically differentiated. The optic lobes are partially differentiated. The infundibulum has a thin wall but contains in its cavity a large mass

of cells. The lateral walls of the midbrain are excessively thick and the lumen of the aqueduct is narrow. The ventral wall of the medulla is irregular in outline in places (section *b*). There are many desquamated cells in the fourth ventricle. The spinal cord is greatly deformed in most regions and in many cases shows no central canal (sections *d* and *e*). In the midthoracic region it is fairly normal in form. The spinal cord does not extend into the rudimentary tail.

*Organs of Special Sense*—The nasal fossæ are patent. The medial wall of each fossa is thin (section *a*). The optic stalks are patent. The pigment layer of the retina is separated from the sensory layer. The latter is not specifically differentiated. A lens rests against the sensory layer (section *a*). The auditory vesicles are simple sacs with dorsal diverticulæ.

*Peripheral Nerves*—The sensory ganglia of the fifth, seventh and eighth, ninth and tenth nerves can be distinguished. The nerves can be followed but a short distance. Spinal ganglia are present in the midthoracic region where the spinal cord is fairly normal in form, but are not present elsewhere.

*Alimentary Canal*—The mouth is patent. The jaws and lips are partially differentiated. The gill slits are patent. The internal gills are rudimentary in form and contain no obvious blood vessels. The operculum is only partially differentiated. There is no opercular cavity in front of the heart. The tracheo-pulmonary anlage is a short branched column of cells. The œsophagus is composed of dense tissue in which several irregular spaces suggest a coiled tube (section *c*). The stomach curves about the left side of the anlage of the liver (see right side of section *d*). The liver is highly differentiated (section *c*) and is connected by ventral and dorsal mesenteries to the walls of the body cavity. The large blood spaces in the liver contain traces of blood. The bile duct is large. The pancreas is not specifically differentiated but its anlage is marked by a mass of yolk cells. The gut curves from left to right in front of the anlage of the pancreas and then turns distally. Beyond the region of the pancreas the gut passes nearly straight back to the anus. The ventral portion is thick and filled with yolk cells as in young larvæ. The anus opens on

the right side of the body between the rudimentary tail and the body cavity (section *f*). The body cavity is greatly distended (sections *d*, *e*, *f*).

*Heart and Blood Vessels*—The heart is a simple S-shaped tube. The ductus arteriosus is patent; the ventricle has very thick walls, a small lumen and no trabeculæ. The sinus venosus and the auricle are thin-walled. There are a few blood corpuscles in the lumen of the heart. Mere traces of blood vessels are visible.

*Genito-Urinary Organs*—The tubules of the pronephros are much dilated. They lie in large spaces which here and there contain a few blood corpuscles (section *d*). The Wolfian ducts are much distended (section *e*).

*Skeleton and Connective Tissues*—The chorda is relatively normal in structure. There is an excessive amount of connective tissue, especially in the region of the head. The cartilages of the head are partly differentiated. There are no auditory capsules.

*Musculature*—The muscles of the head are partially differentiated. The myotomes are relatively fairly well developed (sections *d* and *e*).

### Larva No. 5, Experiment II

*External Form*—The tail curves dorsalward. The abdomen is distended. The head is somewhat irregular in form. The sucker consists of a short projection on the right side of the body.

*Central Nervous System*—Anteriorly there are two small olfactory lobes. The lateral ventricles extend but a short distance into each. The pineal body is round and has a slender stalk. The cells composing the body are more or less scattered, although the outer wall of the body is fairly smooth. The lateral walls of the third ventricle are thick and show a fairly normal differentiation into cells and Randschleier. The hypophysis has a very thin wall. The optic lobes are not differentiated. The fourth ventricle is much distended. The walls of the medulla contain many degenerated pigment cells.

*Organs of Special Sense*—The tissue of the medial wall of each nasal fossa contains many degenerated pigment cells. The pigment layer of the retina is separated from the sensory layer.

Neither optic stalk shows a lumen. The sensory layer of the retina shows much degeneration. The lens rests against it. The auditory vesicles are simple.

*Peripheral Nerves*—The sensory ganglia of the head and the nerve branches may be followed better than in most of the experiment larvæ. The spinal ganglia and nerves are less definite.

*Alimentary Canal*—The mouth is patent and is surrounded by partially differentiated jaws and lips. The internal gills are imperfectly developed. The operculum extends but a short distance posteriorly. The œsophagus is occluded with cells. The tracheo-pulmonary process is short and branched. The œsophagus continues to the left into the stomach. The gut curves ventrally to the right in front of the pancreas, then anteriorly on the right of the liver and then bends back in a posterior direction. The liver is well developed and contains large sinusoidal spaces. A very few blood corpuscles appear to be contained in these spaces. There is a large gall bladder. The pancreas is also well developed. The gut, immediately posterior to the pancreas, is greatly distended and beyond the region of distention exhibits several partial coils. The anus is patent. In the body cavity many multi-nucleated cells can be seen. The walls of the body cavity are excessively thick.

*Heart and Blood Vessels*—The heart is S-shaped. The ductus arteriosus is solid. There is a small lumen in the ventricle. The sinus venosus is thin-walled. In places blood vessels containing a small amount of blood may be seen, but the vascular system is imperfectly developed.

*Genito-Urinary Organs*—The tubules of the pronephros are greatly distended and lie in large spaces in which some blood corpuscles may be seen. The Wolfian ducts are greatly distended near the pronephros but not much more distally. That on the left side is much smaller than that on the right.

*Skeleton and Connective Tissues*—The chorda dorsalis is moderately normal in structure. In the sections it is shrunken. There is an excessive amount of connective tissue, especially in the region of the head and in the wall of the body cavity. The cartilages of the head are fairly well developed.

*Musculature*—The muscles of the head are well differentiated. In places the spinal myotomes are fairly normal; in places the cells composing them are much scattered.

*Skin*—The epithelium shows irregular projecting processes in many places.

#### Larva No. 6, Experiment II, Plate V

*External Form*—The long axis of this embryo is nearly straight. The head appears much crumpled. The mouth is a large cavity bounded by irregular folds of tissue. There is a small semi-circular sucker back of the oral opening.

*Central Nervous System*—The brain is anteriorly much dilated (section *a*) and extends to the anterior extremity of the head. The neural wall is very thin and the central cavity is filled with degenerated cells. The dorsal wall of the forebrain in places is fused to the ectoderm (section *b*). There are no visible traces of a pineal body. The ectoderm is irregularly thickened where it comes in contact with the brain and in some places is very thick. In one place a long column of ectoderm cells extends in between the ectoderm and the brain (right side of section *b*). The dorsal wall of the brain on each side in the region of the eyes is greatly thickened. The midbrain is most irregular in form and gives rise to several vesicular processes of uncertain nature. The walls of the midbrain are thin and the central canal is large. The medulla is most abnormal in form (sections *c* and *d*). The dorsal part of the fourth ventricle is curiously dilated. The spinal cord is more normal in form than the brain although it also is much deformed in places.

*Organs of Special Sense*—The only trace of nasal epithelium is a collection of cells between the dilated pharynx and the ectoderm in the anterior part of the head shown on the right side of section *a*. The eyes are very abnormal. The optic stalk is dilated so as to make a direct opening from the neural canal to the back of the sensory layer of the retina. There is no trace of specific differentiation in this layer (section *b*). Where the optic stalk approaches nearest to the ectoderm a lens has been differentiated. This is still in contact with the ectoderm and consists of

a group of little differentiated ectoderm cells. The auditory vesicles are simple in form, thick-walled, and resemble those newly formed in the embryo.

*Peripheral Nerves*—Mere traces of the sensory ganglia of the cranial and spinal nerves can be seen. Nerve fibers are not well marked.

*Alimentary Canal*—The mouth is a large opening into the pharynx. The opening is irregular in outline and its boundary presents mere traces of a beak and lips. The pharynx is dilated anteriorly (section *a*), but over the cardiac region is flat and bent to conform to the dilated pericardial cavity. The gill clefts nowhere open to the exterior but instead are laterally stopped up by masses of epithelial tissue (section *c*). No gills are specifically differentiated. The œsophagus is filled with epithelial cells. The tracheo-pulmonary process is rudimentary. The œsophagus passes directly back into a primitive intestine. Neither liver nor pancreas seem specifically differentiated, although the anlagen of each are represented by masses of yolk cells (section *e*). The gut consists of a mass of cells which extends in a fairly straight direction from the œsophagus to the anus. Anteriorly it shows some tendency to form convolutions, and in the mid-body region it curves slightly toward the right side of the body. For the greater part of its course no lumen is present.

*Heart and Blood Vessels*—The pericardial cavity is very thick-walled anteriorly but is relatively large. More distally the ventral wall is thin (section *c*). Traces of a heart can be seen (sections *c* and *d*). This forms a slightly S-shaped structure with thin-walled ductus arteriosus and sinus venosus (section *d*) and a more solid ventricle (section *c*). There is no blood in the heart cavity. No definite blood vessels can be made out in the embryo.

*Genito-Urinary Organs*—The tubules of the pronephros are much dilated. The Wolfian ducts can be traced only part of the way to the anlage of the cloaca. In the distal part of their course they are curious flat tubes (section *f*, on each side of body).

*Skeleton and Connective Tissues*—The chorda dorsalis is slightly asymmetrical in places. The connective tissue is excessive, especially in the head. The cartilages of the head are not distinct.



*Musculature*—Mere traces of muscles are found in the region of the head. The more anterior myotomes consist of rounded masses of cells on each side of the chorda but not in contact with this (section *e*). In the center of the trunk and in the tail they are somewhat more normal in form.

*Skin*—Projections of epithelium may be seen on the dorsal margin of the tail (section *g*) and at the side of the ventral wall of the body cavity (section *e*). There are curious subcutaneous vesicles on each side of the head posterior to the sucker. Section *b* shows one of these at the left of the section.

#### Larva No. 7, Experiment II, Plate IV—B

*External Form*—The tail is short and stubby and curves dorsally. The heart is shrunken and irregular in outline. The sucker is apparently fairly normal.

*Central Nervous System*—Two well separated olfactory lobes project forward as far as the nasal fossæ and each is in contact with the medial wall of the corresponding nasal fossa (section *a*). The tissues of the olfactory lobes are partially degenerated. The lateral ventricles are very small. The lateral walls of the third ventricle are thick and partially differentiated (section *b*). The pineal gland projects above the third ventricle and its tissue is partly degenerated. The infundibulum is small and thin-walled. The lateral walls of the mid-brain are thick and project inward so as to nearly obliterate the aqueduct. The hind-brain is relatively normal, although flattened from front to back (sections *c* and *d*). The spinal cord in places is fairly normal, in places the cells from the walls of the neural tube fill or nearly fill the central canal.

*Organs of Special Sense*—The nasal organs are fairly well differentiated. They lie, relative to the brain, posterior to the normal position. The pigment layer of the retina is separated by a space from the sensory layer. The latter is not well differentiated. The optic stalk is not patent but contains no nerve fibers. A lens is present. The auditory vesicles are simple in form.

*Peripheral Nerves*—Cranial and spinal ganglia are moderately well developed. The nerve fibers cannot be readily traced.

*Alimentary Canal*—The mouth is open. Lips and jaws are rudimentary. The pharynx is dilated and contains a sac-like protrusion through its floor from the pericardial cavity (section *c*). The gills are rudimentary and are contained within a cavity on each side which is formed by an opercular fold open behind (section *d*). The œsophagus has a lumen into which irregular vesicular spaces open. The tracheo-pulmonary process is short. The gut passes to the left of the liver and pancreas, then curves across the body in front and finally extends straight back. The lumen is not distinct. Neither liver nor pancreas is well differentiated. The body cavity is greatly dilated.

*Heart and Blood Vessels*—The heart is an S-shaped tube. The ductus arteriosus and ventricle are thick-walled; auricle and sinus venosus are thin-walled. There is a slight amount of blood in the lumen. The pericardial cavity projects into the mouth, pushing the floor of the pharynx ahead so that the heart comes to lie literally in the mouth. The only definite blood vessels are two vessels which appear to be posterior cardinal veins. These are dilated and anastomose with one another in several places.

*Genito-Urinary Organs*—The tubules of the pronephros are greatly dilated. The Wolfian ducts are irregular in form. That on the right side appears to be missing in places.

*Skeleton and Connective Tissues*—The chorda dorsalis is apparently normal. There is an excessive amount of connective tissue. Some of the cartilages of the head are fairly well differentiated.

*Musculature*—Some of the muscles of the head are fairly distinct. The myotomes are moderately normal, although in places the muscle cells are somewhat scattered.

*Skin*—In the region of the head there are especially large masses of projecting epithelium (section *a*).

#### Larva No. 8, Experiment II

*External Form*—The long axis is fairly straight. The general appearance is that of a normal embryo soon after the tail has grown out. The tail, however, is somewhat shrunken. The sucker is fairly normal.

*Central Nervous System*—The olfactory lobes extend forward between the nasal fossæ. The tissue of the olfactory lobes is somewhat degenerated. The pineal gland consists of a rounded hollow vesicle with a short much dilated stalk. The ventral wall of the third ventricle is abnormally thick. The infundibulum is relatively normal. The walls of the midbrain are also fairly normal but contain some degenerated pigmented cells. The optic lobes are beginning to be differentiated. The hindbrain and spinal cord are relatively normal.

*Organs of Special Sense*—The medial walls of the nasal fossæ are thick but contain many pigmented and degenerated cells. The eyes are very abnormal. The optic stalks are greatly distended. The pigment layer of the retina is irregular. The sensory layer consists of a mass of degenerated cells. The lens is differentiated. The auditory vesicles are simple in form.

*Peripheral Nerves*—The sensory ganglia are moderately well developed but the cells are many of them abnormal. Nerve fibers cannot be readily followed.

*Alimentary Canal*—The mouth is open. Lips and jaws are clearly marked, although not highly developed. Gill slits are patent, but the gills are not well developed and seem to contain no blood vessels. Opercular folds extend over the anterior portion of the gill region on each side. The œsophagus has a lumen. The tracheo-pulmonary process is short and branched. The stomach lies at the left of the liver anlage. The gut extends straight back. Neither liver nor pancreas is well developed, although masses of cells indicate their anlagen. No blood spaces are found in the liver.

*Heart and Blood Vessels*—The heart consists of an S-shaped tube, but the walls are not normally differentiated. There is some blood in the ductus arteriosus. There are apparently a few blood vessels present but there is no well developed vascular system.

*Genito-Urinary Organs*—The tubules of the pronephros are slightly dilated.

*Skeleton and Connective Tissues*—The chorda dorsalis is relatively normal. The cartilages of the head are fairly well developed.

There is a slight increase over the normal amount of connective tissue in the body. The pigment cells are abnormally scattered about.

*Musculature*—The muscles are fairly well differentiated in the head. The more anterior of the myotomes are fairly normal but in the posterior half of the embryo they are not well developed.

*Skin*—In many places the skin shows abnormal outgrowths especially about an irregular opening into the body cavity.

#### SUMMARY AND CONCLUSIONS

Toad spermatozoa removed from the body begin to lose both motility and fertility within half an hour. Both motility and fertility last much longer on cool than on warm days and somewhat longer in unexposed than on spermatozoa exposed to the Roentgen rays. On cool days the power of fertilizing lasts in some of the spermatozoa over two hours. When only ten or fifteen per cent of the control eggs are fertilized as a rule few or none of the eggs placed with the exposed sperm are fertilized.

When only a few eggs are fertilized by the exposed sperm as a rule these eggs do not develop beyond the gastrula stage, but occasionally one may develop into an abnormal tadpole.

When the spermatozoa have been well exposed to the rays and yet are still capable of fertilizing a considerable number of eggs, the eggs thus fertilized develop at first apparently normally or even better than the control, but beyond the gastrula stage the development begins to become retarded and at the time of hatching, as the tail begins to grow out, marked deformities appear in the larvæ. These deformities are visible externally and are still more striking when the internal structure is examined. The illustrations given on Plates II to V illustrate these deformities more readily than they can be described in words. While they vary considerably there are certain features characteristic of most of the tadpoles.

#### *General Development*

Growth of the tadpole is inhibited beyond the stage which intervenes between hatching and the time when it should begin to

swim. Thus when the control tadpoles of the same age as the experiment tadpoles are equivalent in general form to the 12 mm. tadpole of the frog described at some length in Marshall's well-known text-book, the development of the control tadpole is, as a rule, more nearly similar to the newly-hatched tadpole of the frog described by Marshall.

### *External Form*

The head is usually abnormal in shape, the anterior end appearing shrunken. The coelom is in many of the tadpoles abnormally distended. The tail is usually short, more or less deformed and is often bent in a dorsal direction.

### *Internal Structure*

The vascular system is little developed in any of the experiment embryos. The heart usually is S-shaped but is rudimentary in form and may have no continuous lumen. In some embryos the wall of the ventricle is thickened by muscle cells but in none are there strong trabeculae in the ventricle. The chief arteries seem in none of the embryos to be completely developed, although in some there are here and there traces of them. The chief veins are likewise in none of the embryos completely developed although in one embryo the cardinal veins are large. In the liver the capillaries are sometimes well, sometimes but slightly developed. There are relatively a very few blood corpuscles in any of the embryos. These lie in some of the scattered vascular anlagen. It is uncertain whether the blood had circulated in any of the embryos, but in some of them it is fairly certain that no circulation was established. In all of the embryos the spaces in the tissues indicate a considerable amount of lymph either free in the tissues or confined in lymph vessels.

Of the central nervous system the brain is the part most constantly and deeply affected, but the spinal cord in many of the embryos is markedly deformed. The abnormalities consist partly of failure of development or tissue differentiation, partly of irregular growth of tissue, pigmentary degeneration of nerve cells and the

filling of the central canal with partially degenerated cells. In one embryo the hind brain and anterior part of the spinal cord are exceedingly rudimentary on one side.

Of the organs of special sense the eye exhibits the greatest deformities. The nose and ear are as a rule rather rudimentary than markedly deformed. The eye, however, usually shows a patent optic stalk connecting with a space between the pigment and sensory layers of the retina, a lack of differentiation in the sensory layer, and a more or less highly differentiated lens resting against the sensory layer.

The abnormalities in the alimentary canal are exceedingly variable and may affect any or all parts. The mouth is in all instances patent, the lips and jaws rudimentary. The pharynx and gills vary much in structure in the different embryos. As a rule there are traces of internal gills and of the opercular folds but the gills, owing to lack of development of the vascular system, are rudimentary. The œsophagus is patent in some, closed in other of the embryos; the stomach as a rule lies at the left of the anlagen of the liver and pancreas. The latter structures are seldom highly developed. The rudiments of the lungs are slightly developed. The intestines may be more or less coiled, but are in none of the embryos highly developed and in some are very rudimentary. In many embryos the abdominal cavity is greatly distended while the gut is rudimentary.

The pronephric tubules are usually greatly swollen in places and this dilatation is also frequently found in the Wolfian ducts. There are seldom distinct traces of the metanephric tubules.

The myotomes, when not well developed, usually consist of muscle cells somewhat scattered about in the surrounding mesenchyme. The muscles of the head are usually more or less differentiated.

The mesenchyme of the embryos is considerably greater in amount than in normal tadpoles. The cells seem to be spread apart by fluids in the tissues. The cartilages of the head and the chorda dorsalis are relatively normal.

The ectoderm in most of the tadpoles shows in places outgrowths of an irregular nature. These may be extensive villus-like pro-

cesses. In one instance marked ingrowth of processes from the ectoderm occurred.

The cells of the tissues appear for the most part clear in outline. Many of the cells of the central nervous system seem to have undergone a pigmentary degeneration. Numerous cells in most of the tissues show mitotic figures. I have been unable satisfactorily to determine whether or not there are abnormalities in these figures. There is an abnormal number of cells with two or more nuclei. A striking feature of the experiment embryos is the irregular distribution of the pigment cells. They are much more irregularly distributed through the tissues than in the normal embryos.

There is a striking resemblance between tadpoles which develop from ova fertilized by sperm exposed to the Roentgen rays and the tadpoles exposed directly to radium irradiation by Schaper<sup>81</sup>. This shows clearly that injuries produced in nuclei may be carried through many generations of cells in an individual and finally give rise to deformities corresponding with those due to direct irradiation. Bohn<sup>82</sup> found that the rays of radium rapidly enfeeble or kill the sperm of *strongylocentrotus lividus*, but that the eggs appear more fertile after exposure. He does not describe the effect of exposure of the germ cells on subsequent development.

Herbst<sup>83</sup> found by treating the sperm of sea-urchins with fresh water, alkalies and potassium-free salt water and then fertilizing ova of a different species with the sperm thus injured that the ova sometimes developed as if injured but that there was no evidence that the specific hereditary factors transmitted by the spermatozoa were altered. Further studies are necessary to determine if the hereditary factors carried by the sperm may be specifically influenced by irradiation. D. T. Macdougall<sup>84</sup> has shown that in some plants mutations may be produced by injecting radium preparations, sugar solutions and solutions of calcium nitrate and of zinc sulphate into the ovaries. This most important work sug-

<sup>81</sup>Schaper: *Anat. Anzeiger*, xxv, p. 298, 1904. Levy: *Archiv f. Entwicklungsmechanik*, xxi, p. 130, 1906.

<sup>82</sup>G. Bohn: *Comptes Rendus de l'Acad. des Sciences, Paris*, cxxxv, p. 1012, 1085, 1903.

<sup>83</sup>Herbst: *Archiv für Entwicklungsmechanik der Organismen*, xxi, p. 293, 1906.

<sup>84</sup>MacDougall: *The Popular Science Monthly*, September, 1906, p. 16.



gests that the hereditary factors contained in spermatozoa might be so altered as to produce specific variation in the individuals springing from ova which they fertilize.

The effects of altering the normal course of development of vertebrates by electrical, magnetic, chemical or mechanical agents applied to the whole organism, have been shown by Dareste,<sup>85</sup> Féré,<sup>86</sup> Roux,<sup>87</sup> and many others, to be seldom confined in a specific way to an organ or group of organs, although some organs, like those composing the nervous system, are especially sensitive to all such factors. The experiments with irradiation show that although some tissues are much more susceptible to the rays than others, there are wide differences in the effects of the rays on different individuals.

In conclusion, I desire to express my thanks to my colleague, Professor B. W. Snow, for the use of the Roentgen ray apparatus belonging to the Department of Physics, and for his aid in conducting the exposures.

#### EXPLANATION OF PLATES I TO V.

On these plates there are represented outlines of the external forms and transverse sections through the body of one control and five experiment larvæ. Descriptions of the larvæ represented are to be found in the text as follows:

Plate I, p. 21

Plate II, p. 24

Plate III, p. 27

Plate IV—A, p. 31

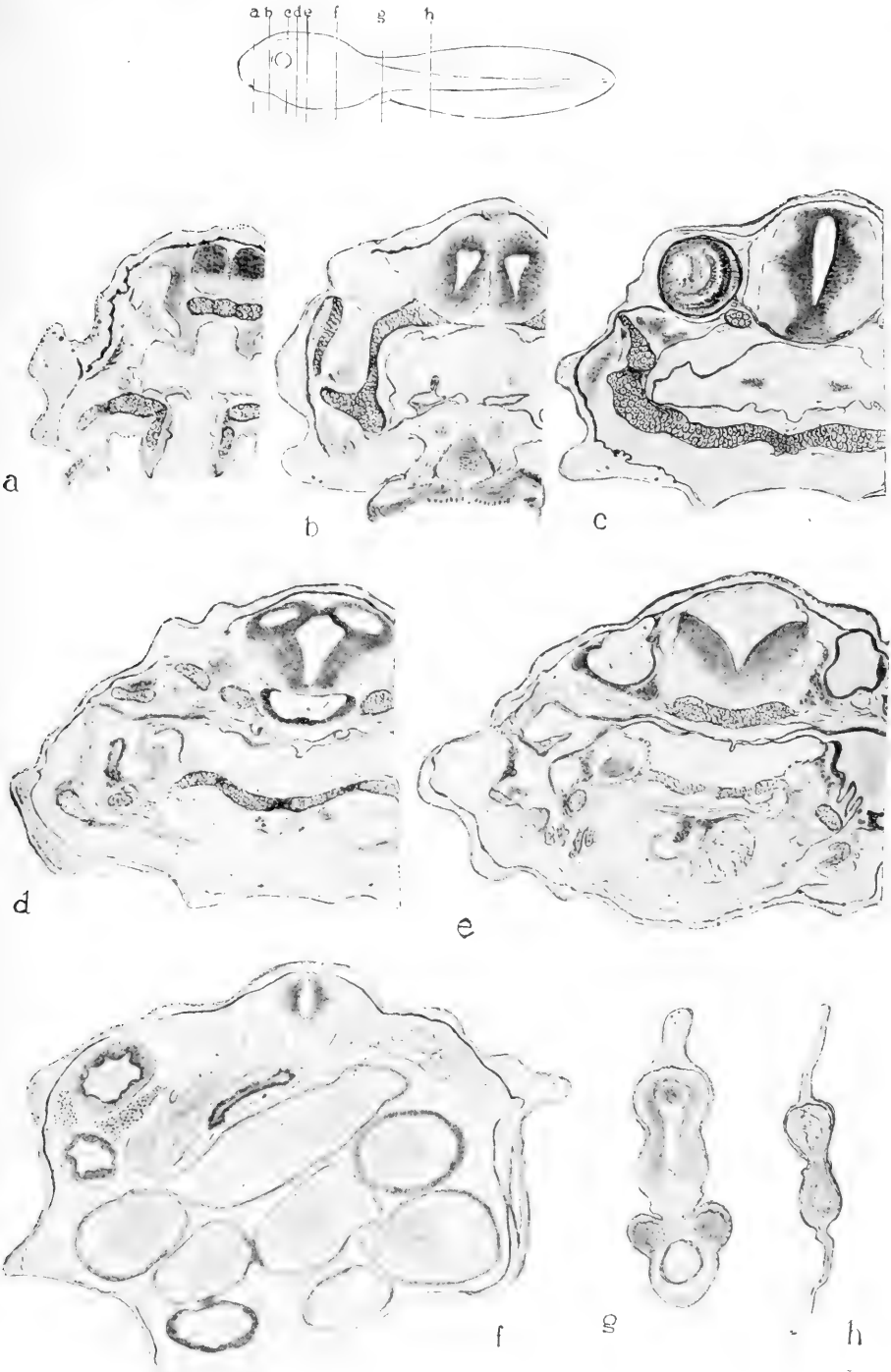
Plate IV—B, p. 37

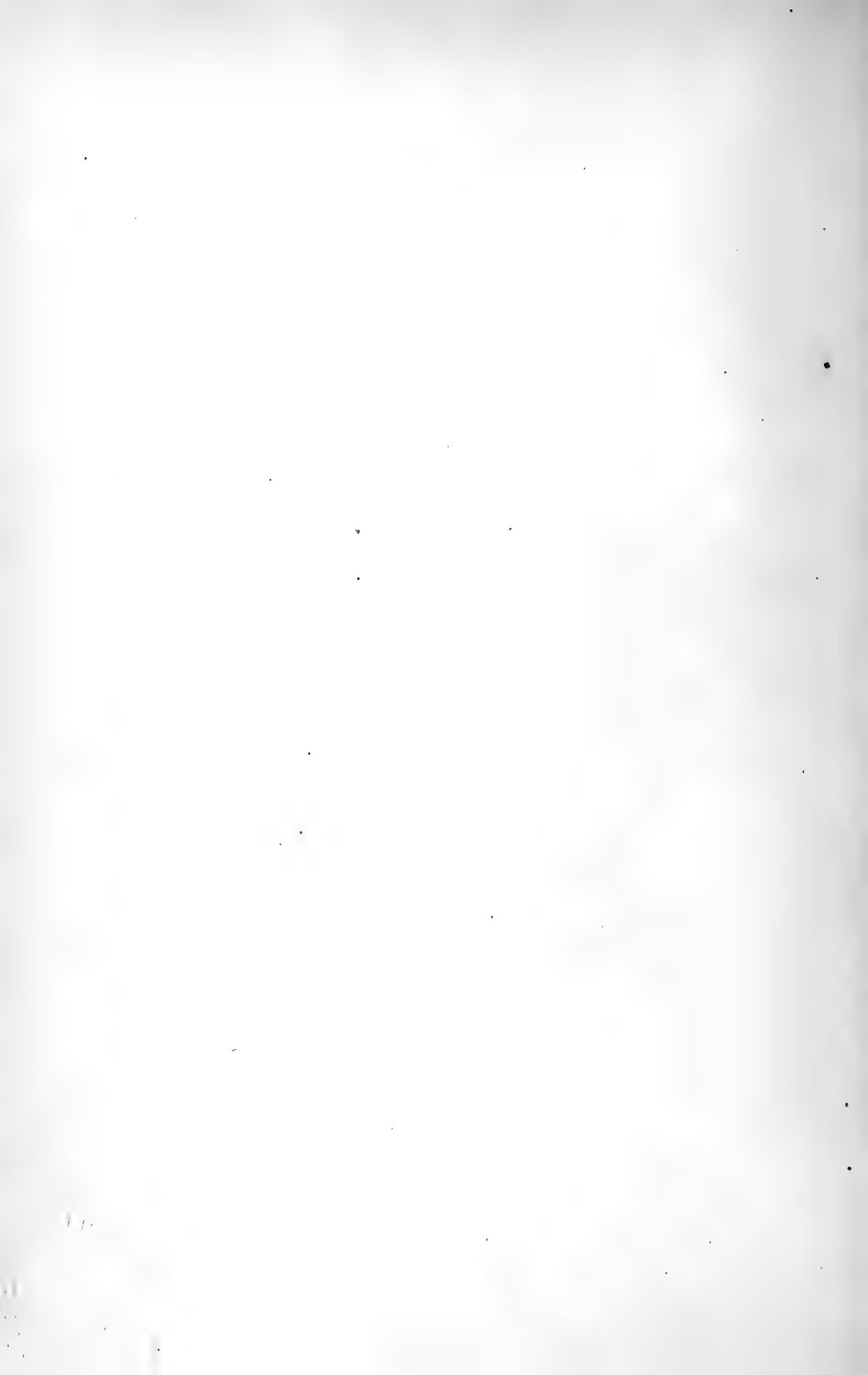
Plate V, p. 35

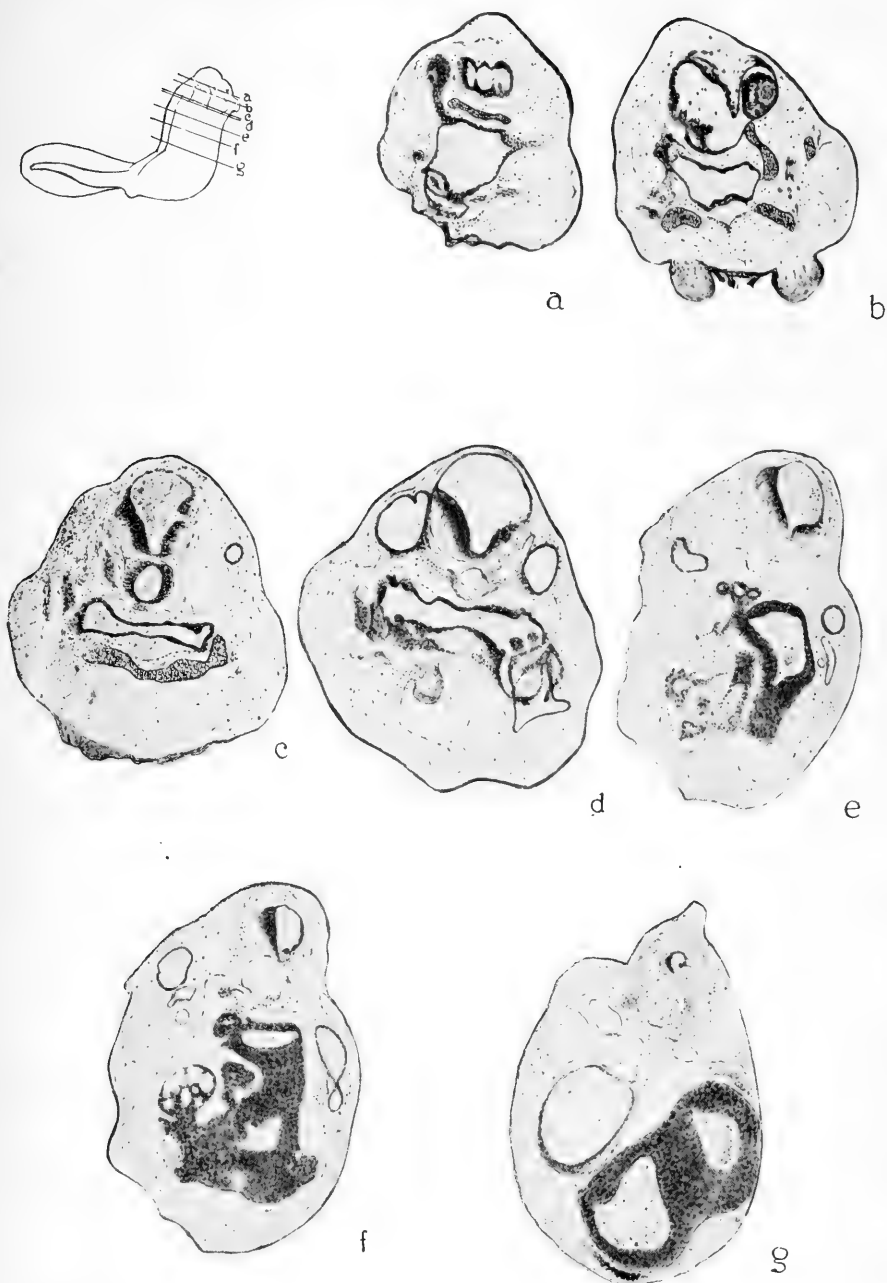
<sup>85</sup>Dareste: *Recherches sur la production artificielle des monstruosités*. Paris 1891.

<sup>86</sup>Féré: *Comptes Rendus de la Société de Biologie*, 1893-1905

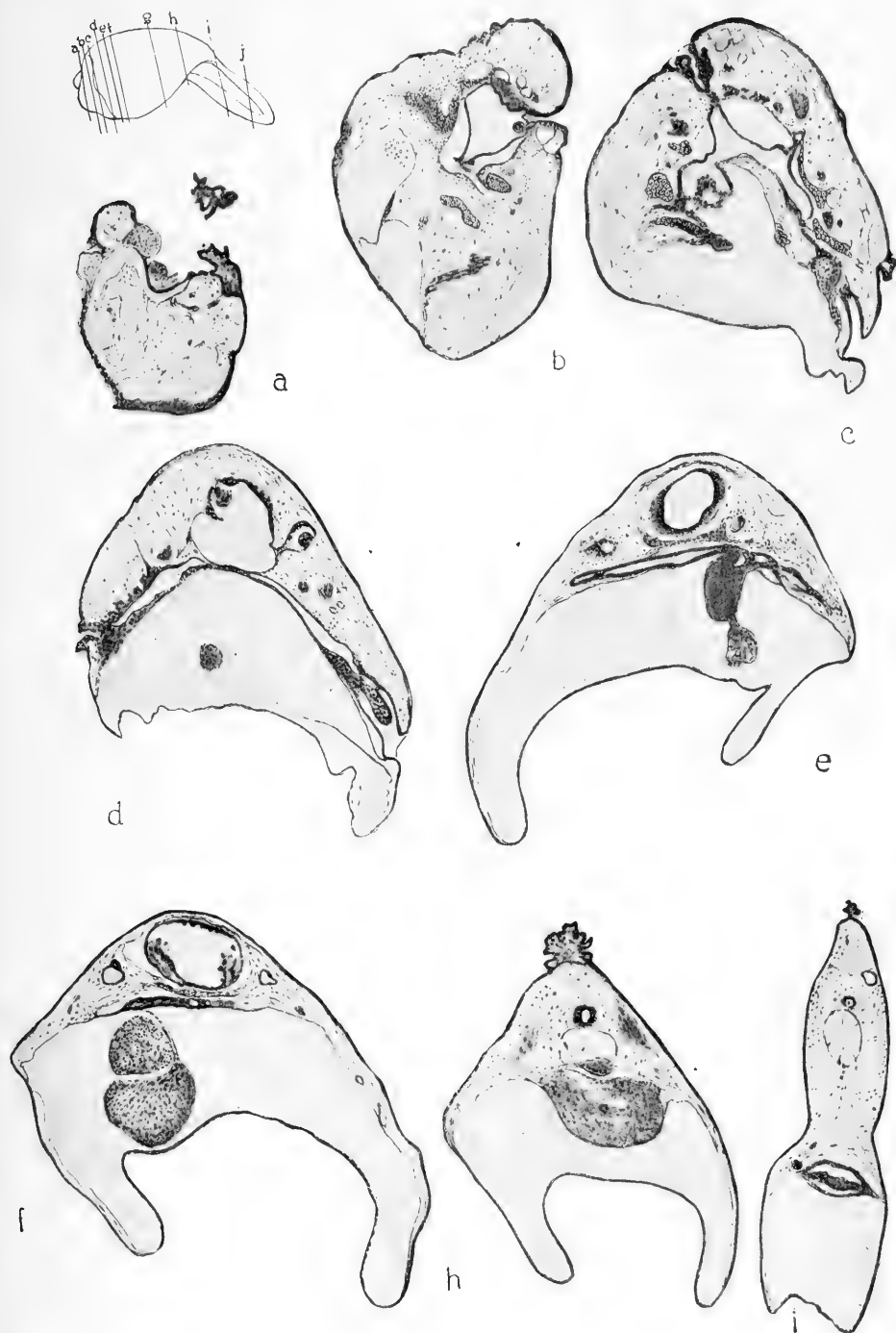
<sup>87</sup>Roux: *Gesammelte Abhandlungen über Entwicklungsmechanik der Organismen*, 1895.



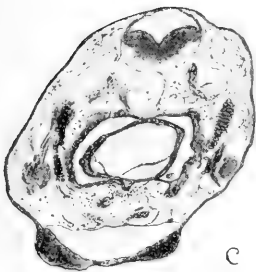
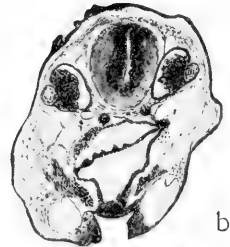
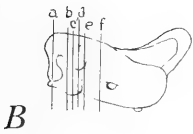
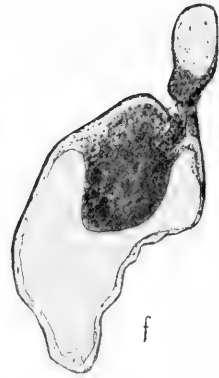
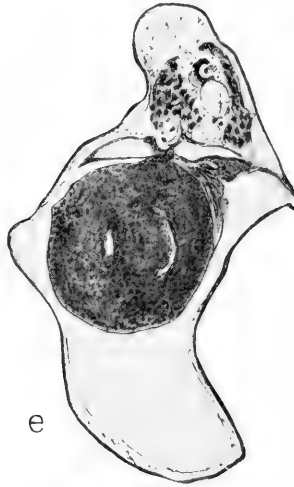
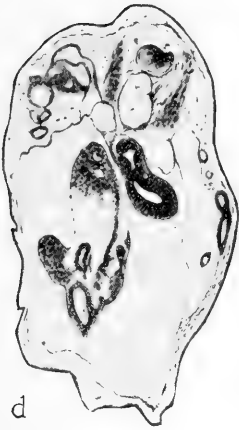
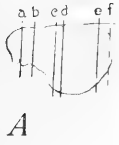




















# AN ECOLOGICAL AND EXPERIMENTAL STUDY OF SARCOPHAGIDÆ WITH RELATION TO LAKE BEACH DEBRIS<sup>1</sup>

BY

WILLIAM B. HERMS

WITH SEVEN FIGURES

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## I INTRODUCTION

The organic débris of Cedar Point beach near Sandusky, Ohio, as observed for the summer months consists principally of dead fish, though salamanders and carcasses of birds are occasionally cast up. In addition to these there is always a greater or lesser drift of *insects* after each high surf. This insect drift consists largely of the exuviae (not useful to scavengers because of lack of organic matter) of the abundant May flies (Ephemeroidea), also numerous dead and some living individuals of the same order. Great windrows of these insects together with their exuviae may often be seen reaching for miles along the beach.

Among other aquatic insects carried in by the surf, the more conspicuous forms are: Hydrophilidae, Dytiscidae (2 species), and Belostomatidae (3 species). Stag beetles (Lucanidae), May beetles (Scarabaeidae), Ground beetles (Carabidae), Dragon flies (Odonata), Caddice flies (Phryganeidae), Crickets (Gryllidae), a few butterflies (chiefly *Anosia plexippus*) and moths (Lepidoptera) are all

<sup>1</sup>Presented to the Faculty of the College of Arts, Philosophy and Science of the Ohio State University as partial requirement for the degree of Master of Arts, June, 1906.

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generally a part of the drift. Needham ('00) gives a very comprehensive list of insects found in the insect drift on the shores of Lake Michigan, however, under extraordinary conditions.

Every high surf brings in débris of the above general character which is usually proportional to the intensity of the surf. There are *always* a few fish being cast up on the beach somewhere, though the writer has walked miles on three successive days without finding a single dead fish on the sand or in the edge of the water. Thus it can be seen that the scavenger insects of the beach must depend on the surf for their food. This is especially true for this location, because of the peculiar form of Cedar Point, which extends in a northwesterly direction into Lake Erie for about six miles as a narrow arm of sand not more than one-fourth of a mile wide at the widest point and that at the tip. It is the impression of the writer that the flesh flies and other scavengers would not readily retreat to the mainland for food when there is a lack of it on the Point.

In order to gain a fair estimate of the number of fish in particular that are thrown up by the waves from time to time, several early morning trips were taken along the beach. All fish which had been washed up during the night previous, for the distance of a mile, were weighed with a balance. The first trip was made July 9, 1905. The surf had begun to rise late in the afternoon of the previous day and was running fairly high by 5 o'clock, when dead fish began to appear. The weighing was begun at 4 o'clock, a. m., and gave the following results:

*Fish cast up during the night of July 8, 1905, from about 5 p. m. to 4 a. m., July 9, 1905, surf flowing fairly high all night. Distance—One mile of beach*

Saugers .....	14
Minnows .....	10
Bass and perch .....	15
Mooneye .....	2
Cat .....	3
Carp .....	1

Total .....45

Total weight, 4.65 kilograms.

This gives an average of 103.3 grams. The result on the whole was disappointing, fewer fish having been cast up than during similar surfs.

A second weighing trip over the same ground was made about two weeks later, conditions being similar. The results of this trip are as follows:

Perch and small-mouth bass	441
Saugers	18
Minnows	50
Bass	12
Cat	10
Miscellaneous	7

Total ..... 538

Total weight, 20.38 kilograms.

The average 37.9 grams is low, but this is due to the fact that practically all of the perch and small-mouth bass were young, ranging in weight from 14 to 42 grams. The lake storms appear at quite regular intervals during the summer, which allows a period between each high surf for a complete cleaning up by the scavenger insects. For this reason it is possible to get only those fish on record which are newly washed up.

It would make an interesting and no doubt profitable investigation to ascertain the cause of death to the large number of young perch and bass which are cast up at times during the high surfs.

With a beach so well scattered with dead fish as to show 538 to a mile, and every reason to believe that this was not an exceptional stretch of beach on this particular morning, one has some conception of the work to be done by Nature's scavengers. A walk along this same mile of beach about three days after a storm can but impress one with the effective work of the scavenger insects. An examination will show that the many fish carcasses have been reduced to mere shells, which are comparatively odorless.

Below is a list of the scavengers most active in the removal of organic beach débris. This list is not intended to be a complete one, there are other species of both Diptera and Coleoptera and also other insects active in the work though in a very small degree.

*Diptera*<sup>2</sup>

- Fam. Sarcophagidæ  
*Lucilia cæsar* Linné  
*Compsomyia macellaria* Fabr.  
*Sarcophaga sarraceniae* Riley  
*Sarcophaga assidua* Walker

*Coleoptera*

- Fam. Staphylinidæ  
*Creophilus villosus* Grav.  
Fam. Scarabæidæ  
*Trox scabrosus* Beauv.  
Fam. Silphidæ  
*Silpha americana* Linné  
*Necrophorus orbicollis* Say.  
*Necrophorus vespilloides* Hbst.  
*Necrophorus tomentosus* Web.  
Fam. Dermestidæ  
*Dermestes vulpinus* Fab.  
*Dermestes caninus* Germ.  
Fam. Histeridæ  
*Saprinus pennsylvanicus* Payk.  
*Saprinus lugens* Er.  
Fam. Calandridæ<sup>3</sup>  
*Sphenophorus ochreus* Lec.  
*Phytonomus punctatus*

Of the above, the flies are the chief agency, and it is a brief study of these that we shall enter upon.

II HABITS AND LIFE HISTORIES OF *LUCILIA CAESAR*, THE COMMON GREEN FLESH FLY; *COMPSOMYIA MACELLARIA*, THE SCREW-WORM FLY; *SARCOPHAGA SARRACENIAE*, THE COMMON LARGE GRAY FLESH FLY; AND *SARCOPHAGA ASSIDUA*, A SMALL GRAY FLESH FLY

*Habits of Adults*

On emergence from the pupal cases, after the wings are sufficiently dry, the first impulse is to seek food. It seems to be the object of each individual to seek its own nourishment, since each fly takes flight alone. These insects are never seen flying about in aggregation in quest of food. *Sarcophaga sarraceniae* is rarely found in large numbers about a carcass, while *Lucilia cæsar* and *Compsomyia* may be very numerous.

<sup>2</sup>Diptera kindly identified by Prof. J. S. Hine, of Ohio State University.

<sup>3</sup>Accidental.

The presence of food is detected in a remarkably short time, and this can only be accounted for on the assumption of a very acute sense of smell. Comparatively fresh fish were exposed where no flies were to be seen, and in ten or fifteen minutes many flies were hovering about the food and some eggs had already been deposited.

That the compound eyes so prominent in the *Sarcophagidæ* are of importance in orientation we are reasonably certain. If these insects were deprived of their eyesight, food would probably be found with difficulty. In several cases the eyes of *Sarcophaga sarracenix* were painted with india ink, affecting the flies in a manner similar to that of animals whose semi-circular canals are disturbed. Orientation was almost completely lost for a time. On placing the individuals on their backs, they were barely able to right themselves after frantically using both legs and wings. They crawled about on the table in an aimless manner, or on the writer's fingers. After a few minutes they flew slowly away, buzzing noisily, passing over several pieces of fish placed on a table. Their flight was directly toward a window, which they struck with a thud. From this it would seem that the light was not perfectly excluded. No doubt, much of the disturbance above mentioned was due to the penetration of the india ink.

It appears that the adults prefer the fresher food; fresh fish or fish newly cast up being attacked more readily than those having been allowed to dry. This is readily explained because of the greater abundance of liquid food on the bodies of the fresher specimens, and also because of the more pronounced ("fishy") smell of such specimens. On several occasions a fish that had been allowed to dry for a day or two was laid outside, and each time no eggs nor larvæ were deposited thereon, and the fish dried up in the sun. Under natural conditions this would probably not occur.

*Sarcophaga sarracenix* is rarely found in large numbers about a carcass, while the screw-worm fly is most abundant nearer the water and on larger carcasses. *Lucilia cæsar* is found in the majority on large or smaller carcasses farther away from the water and on the small ones near the water.



*Egg Deposition*

*Lucilia cæsar* deposits eggs in irregular masses on the softer portions of the fish, *e. g.*, around the eyes, around the anus, between the gills, on an abrasion, or on the underside of the carcass. This is due to the presence of much liquid food at these particular portions, which the adults suck up while depositing eggs. Whether the deposition of eggs is associated with the stimulation of the food within the alimentary canal of the female is discussed in Chapter VI. The deposition of eggs on these softer portions is, however, an adaptation favorable to the larvæ, since they can thus immediately gain easy access to the body cavity. The gill slits offer good receptacles for eggs, and into these they are pushed by means of the protruded abdominal segments, the open mouth and opercles of the fish affording a good entrance.

Eggs are also commonly deposited on the upper side of the carcass while the fly excitedly flits about sucking the juice. In this way great masses accumulate on the large fish, sometimes one mass growing to the size of a walnut. Several times the mouth and gills were masked with a cloth to watch the effect. This resulted in eggs being laid on the cloth and even on the loose ends of the string with which the mask was tied.

One female was observed to remain in the same position about six minutes, with abdominal segments pushed into a fold of the cloth mask just mentioned. At the end of this time between ninety and a hundred eggs were laid. From among a number of flies taken on a dead fish, a large female (72 mg. in weight) was dissected to ascertain the number of eggs contained within the ovaries. Two hundred and forty-seven fully developed eggs were taken out and the dissected ovaries showed no trace of immature ova. The great weight and distended condition of the female would indicate that few if any eggs had been deposited. How the above number compares with the normal number of ova produced by a single female of *Lucilia cæsar*, the writer cannot say. This matter should have further investigation.

*Sarcophaga sarracenix* deposits living young and deposits them anywhere on the carcass or even near it, compelling the young

larvæ to find a suitable place of entrance. Eighty-two living larvæ were taken from one female.

*Compsomyia macellaria*, the screw-worm fly, deposits very minute living young, but is careless about placing them on the fish. This habit of bringing forth living young seems to be exceptional in this region for the species. From thousands of eggs promiscuously collected, not a single screw-worm fly was reared; all were *Lucilia cæsar*, and all observations in the field resulted in seeing living young extruded. Prof. J. S. Hine, of Ohio State University, reports that he has seen *Compsomyia* deposit eggs and that he has also reared them from eggs at Cedar Point.

### *Larval Habits*

The young larvæ, when hatched or extruded, at once eat into the softer parts, attacking the viscera and later consuming the muscular portions. The fish is eaten clean to skin and bone, the skin remaining as a mere shell; this, too, would be eaten to the scales were the entire surface sufficiently moist. This is evident because the portion of skin nearest the earth, where it is moist, is invariably eaten away, leaving a hole in the under side, which incidentally allows a concealed means of escape during migration.

Migration depends wholly on the food supply. If the fish is large enough and the number of larvæ is not too great, migration takes place in from two and a half to three days, during which time the larvæ have reached their full growth. If the number of larvæ is large in proportion to the fish, migration takes place earlier. This phase of the subject is treated below under the head of "Over and Underfeeding."

On leaving the remains, the larvæ immediately burrow into the sand below or close by the fish. The great majority burrow just beneath, going down two to six inches into the sand and remaining there temporarily. This migration may take place any time during the day or night, though the tactics vary for these periods. Burrowing temporarily just beneath the fish carcass during the day not only affords protection from the intense heat of the sun but also from birds. On cloudy days when migration sometimes takes place away from the fish, the sandpipers, in numbers, feed on the

plump migrating larvæ. Since these birds are quite numerous along the beach one can readily see that this would be a potent factor in extermination, and some means of protection is very advantageous. During the night, or when the sand is cooled, migration from beneath the remains takes place, and it is then that the larvæ travel a greater distance—fifteen, twenty feet and over, and then again burrow. Larvæ that were kept indoors in boxes were observed to repeat this performance several nights in succession, each time burrowing for the day. The sand in the laboratory was not heated by the sun, yet the larvæ followed their normal habit and were characteristically active by night.

Ants are a minor source of destruction to the larvæ that migrate from fish which have been dragged a distance away from the water. Live larvæ, wriggling frantically, are carried away by these little marauders.

At noon of August 10, 1905, several dozen larvæ were found lying dead upon the sand, within a radius of about five feet from a fish. The sand was extremely hot, about  $140^{\circ}$  just below the surface, and the larvæ had been literally baked. What induced this attempt to migrate from the fish at this time is a question.

### *Pupation*

The interval between migration and pupation varies. With individuals reared indoors this interval varies with the degree of moisture—extreme moisture retarding pupation, as also does extreme dryness. A certain amount of moisture is necessary for pupation; therefore, a small amount of water was added to the sand in which the indoor individuals were kept. Temperature probably also affects this stage. All observations were made during the summer months, consequently what happens in spring and autumn later than the middle of September is still open for investigation. The deep loose sand of the beach affords a uniform condition for the burrowing larvæ and for pupation. This condition, together with the food supply, also comparatively uniform, would naturally cause less variation in the life histories of species here than in parts remote from the beach.

The pupal period is quite regular as far as observed; the duration of this stage varies, however, with the species.

The emergence of the imagines from their pupal cases is interesting. With the great blister-like frontal sac, not unlike a tiny balloon attached to their heads, the case is burst and gradually the body is withdrawn, much as a person might extricate himself from a closely fitting tube. All the while the sand particles are



Fig. 1 Cut showing the manner in which screw-worm flies cling to the beach grasses (*Ammophila* and *Panicum*) after emerging from the pupa cases. Notice that the heads are mostly directed downward.

thrown aside by the rhythmically inflated sac. Slowly, pull after pull, the imago passes upward through the sand, and emerges at the surface. After a moment of rest, it starts for the nearest grass stem; up this it crawls in apparent haste, and there it remains to unfold its wings.

The accompanying plate shows the manner in which the screw-worm fly clings to the stems and blades of the beach grasses

(*Ammophila* and *Panicum*), while spreading and drying its wings. When a high surf washes up a big carp into the tall grass, the larvæ after feeding on the carcass, migrate nearby and pupate. When the imagines emerge the grasses are immediately resorted to. This accounts for the presence of veritable swarms of flies in a restricted area with no carcass near. Closer investigation will almost invariably result in finding the bones and scales of some large fish in close proximity.

Most imagines reared in the laboratory emerged early in the morning at the first break of day. In one instance a dozen pupæ were kept in one vial and out of these, seven adults emerged within two minutes.

### *Life Histories*

*Lucilia cæsar* Linné (the common green flesh fly): Eggs of this species are cylindrical, rounded at both ends and slightly curved, smooth and white. The average weight of one egg is about 0.1 mg.<sup>4</sup>

Young larvæ hatch in from eight to eighteen hours, depending on the time of day the eggs are deposited. If deposited toward noon the time will be lengthened, since observations show that in such cases larvæ do not emerge until the following morning together with larvæ coming from eggs deposited any time during the afternoon. This period also undoubtedly varies with the age of the ova at extrusion; *i. e.*, depends on the length of time the ova are retained within the female.

The actual feeding period of the larvæ varies as already indicated from two to two and a half days and over. The interval between migration and pupation varies from two to four days and over, but the actual period of pupation is more constant—about eight days. In the region studied all of the above periods are generally quite regular, so that we may consider the period of development from egg to imago as covering about *fifteen days*, varying a day either way.

*Sarcophaga sarracenix* Riley: This common large gray flesh fly

<sup>4</sup>This weight was obtained by weighing two sets of fifty eggs, four or five hours old. The weight of each set was 5 mg., and by throwing the two sets together 10 mg. resulted. Later five sets of twenty eggs were treated in the same manner, resulting in a like average.

deposits living young. The average weight of newly extruded larvæ, before any food has been taken, is about 0.2 mg.<sup>5</sup> The growth of the larvæ is very rapid, and the feeding period is about the same as *Lucilia cæsar* (two to two and a half days), but pupation takes place more regularly. The interval between migration and pupation is about three days, and the pupal period covers about thirteen days. Thus the period of development for this species is from *eighteen to nineteen days*.

*Compsomyia macellaria* Fabr.: The screw-worm fly is very abundant along the beach, far outnumbering *Lucilia cæsar*. Ordinarily living young are deposited. These can be distinguished from *Sarcophaga sarracenix* larvæ by their small size and lack of the prominent dark coloration of the head. The complete larval period requires about five days, the pupal period about four days, thus giving a very short period of development, namely, *about nine days*.

*Sarcophaga assidua* Walker: Of this species only two adults were reared. This small gray flesh fly, strongly resembling the house fly in size and appearance, has a larval period of five and six days for the two reared, and a pupal period of seven days, giving a total of from *twelve to thirteen days* for development.

### III NORMAL GROWTH OF FLIES THROUGH LARVAL AND PUPAL STAGES

In taking up the study of growth of the species below named, it was decided that weight is the most convenient and most readily applied method of measurement for these forms. As weight has been the basis of measurement for observations made by other investigators, it also forms a readier means for comparison.

Growth is best represented graphically by means of curves, and below is thus shown the course of growth of *Lucilia cæsar* and *Sarcophaga sarracenix* in terms of weight in milligrams.

The eggs of *Lucilia* for this experiment were collected and weighed on the afternoon of July 12, 1905. The larvæ emerged early July 13, when the larvæ of *Sarcophaga sarracenix* were

<sup>5</sup>This weight was obtained by weighing two sets of ten on August 31, 1905, which gave a total of 2 mg.; throwing the two sets together gave 4 mg. for the twenty larvæ.

started. It was not part of the original plan to trace the course of growth of this latter species, but when one of the females deposited a quantity of larvæ on several pieces of unprotected flesh,

TABLE I

*Showing larval growth in six sets of Lucilia Cæsar, including maximum weight at migration. A copy of a portion of the sheet used for tabulation of data*

1				2				3			
1905 July			mgs.	1905 July			mgs.	1905 July			mgs.
		(10)	(1)			(10)	(1)			(10)	(1)
12	7.30 p.m.	20 eggs	2	12	7.30 p.m.	20 eggs	2	12	7.30 p.m.	20 eggs	2
		(10)	(2)			(10)	(2)				
13	9.45 a.m.	20 larvæ	4	13	10.00 a.m.	20 larvæ	4	—	—	—	—
		(10)	(1.3)			(10)	(1.3)				
13	11.45 a.m.	15 larvæ	2	13	12.00 a.m.	15 larvæ	2	13	12.30 p.m.	10 larvæ	2
13	1.45 p.m.	10 larvæ	5	13	2.00 p.m.	10 larvæ	5	—	—	—	—
13	3.45 p.m.	10 larvæ	6	13	4.00 p.m.	10 larvæ	5	13	5.30 p.m.	10 larvæ	13
13	5.45 p.m.	10 larvæ	9	13	6.00 p.m.	10 larvæ	8	—	—	—	—
13	7.45 p.m.	10 larvæ	13	13	8.00 p.m.	10 larvæ	10	—	—	—	—
13	9.45 p.m.	10 larvæ	17	13	10.00 p.m.	10 larvæ	18	—	—	—	—
13	11.45 p.m.	10 larvæ	26	13	12.00 p.m.	10 larvæ	23	—	—	—	—
14	1.45 a.m.	10 larvæ	35	14	2.00 a.m.	10 larvæ	35	—	—	—	—
14	3.45 a.m.	10 larvæ	49	14	4.00 a.m.	10 larvæ	38	14	3.30 a.m.	10 larvæ	64
14	5.45 a.m.	10 larvæ	61	14	6.00 a.m.	10 larvæ	52	—	—	—	—
14	7.45 a.m.	10 larvæ	73	14	8.00 a.m.	10 larvæ	65	—	—	—	—
14	9.45 a.m.	10 larvæ	89	14	10.00 a.m.	10 larvæ	82	—	—	—	—
14	1.45 p.m.	10 larvæ	145	14	2.00 p.m.	10 larvæ	173	14	1.30 p.m.	10 larvæ	178
14	5.45 p.m.	10 larvæ	230	14	6.00 p.m.	10 larvæ	208	—	—	—	—
14	9.45 p.m.	10 larvæ	248	14	10.00 p.m.	10 larvæ	270	14	11.30 p.m.	10 larvæ	310
15	1.45 a.m.	10 larvæ	297	15	2.00 a.m.	10 larvæ	325	—	—	—	—
15	5.45 a.m.	10 larvæ	335	15	6.00 a.m.	10 larvæ	330	—	—	—	—
15	9.45 a.m.	10 larvæ	359	15	10.00 a.m.	10 larvæ	371	15	9.30 a.m.	10 larvæ	*417
		(10)	(386)			(10)	(404)			(10)	(394)
15	3.45 a.m.	5 larvæ	193	15	4.00 p.m.	5 larvæ	202	15	7.30 p.m.	5 larvæ	197

\*Error (?)

these were taken for the purpose and eventually furnished an excellent comparison.

Ten sets of larvæ were started at the same time, twenty in a set for several hours until they were larger when the number was

reduced to ten larvæ in a set. Six of these sets were *Lucilia* and the remaining four *Sarcophaga*. Weighing began shortly after the larvæ emerged from the eggs, July 13, and continued at regular intervals during the entire period of development. Eggs and larvæ were first weighed at extrusion.

TABLE I—Continued

4				5				6			
1905 July			mgs.	1905 July			mgs.	1905 July			mgs.
		(10)	(1)			(10)	(1)			(10)	(1)
12	7.30 p.m.	20 eggs	2	12	7.30 p.m.	20 eggs	2	12	7.30 p.m.	20 eggs	2
		(10)	(2)			(10)	(1.3)				
13	10.30 a.m.	20 larvæ	4	13	10.45 a.m.	15 larvæ	2	—	—	—	—
						(10)	(2.7)			(10)	(3.3)
13	12.30 p.m.	10 larvæ	2	13	12.45 p.m.	15 larvæ	4	13	1.00 p.m.	15 larvæ	5
13	2.30 p.m.	10 larvæ	5	13	2.45 p.m.	10 larvæ	5	—	—	—	—
13	4.30 p.m.	10 larvæ	6	13	4.45 p.m.	10 larvæ	5	—	—	—	—
13	6.30 p.m.	10 larvæ	14	13	6.45 p.m.	10 larvæ	7	13	6.00 p.m.	10 larvæ	8
13	8.30 p.m.	10 larvæ	19	13	8.45 p.m.	10 larvæ	9	—	—	—	—
13	10.30 p.m.	10 larvæ	31	13	10.45 p.m.	10 larvæ	10	13	11.00 p.m.	10 larvæ	23
14	12.30 a.m.	10 larvæ	35	14	12.45 a.m.	10 larvæ	15	—	—	—	—
14	2.30 a.m.	10 larvæ	39	14	2.45 a.m.	10 larvæ	17	—	—	—	—
14	4.30 a.m.	10 larvæ	46	14	4.45 a.m.	10 larvæ	25	14	4.00 a.m.	10 larvæ	45
14	6.30 a.m.	10 larvæ	65	14	6.45 a.m.	10 larvæ	35	—	—	—	—
14	8.30 a.m.	10 larvæ	73	14	8.45 a.m.	10 larvæ	48	14	9.00 a.m.	10 larvæ	84
14	10.30 a.m.	10 larvæ	80	14	10.45 a.m.	10 larvæ	50	—	—	—	—
14	2.30 p.m.	all dead	—	14	2.45 p.m.	10 larvæ	69	14	2.00 p.m.	10 larvæ	151
—	—	—	—	14	6.45 p.m.	10 larvæ	125	14	7.00 p.m.	10 larvæ	223
—	—	—	—	14	10.45 p.m.	10 larvæ	158	14	12.00 p.m.	10 larvæ	284
—	—	—	—	15	2.45 a.m.	10 larvæ	200	—	—	—	—
—	—	—	—	15	6.45 a.m.	10 larvæ	255	15	5.00 a.m.	10 larvæ	350
—	—	—	—	15	10.45 a.m.	10 larvæ	314	15	10.00 a.m.	10 larvæ	374
						(10)	(360)				
—	—	—	—	15	4.45 p.m.	5 larvæ	180	15	3.00 p.m.	10 larvæ	390

Four sets of *Lucilia* were weighed at two-hour intervals for twenty-four hours and the interval increased two hours for each succeeding twenty-four hours until migration. Another set of the same species was weighed at five-hour intervals throughout the larval period to migration, and another set at ten-hour intervals.



TABLE II\*

Showing the average change in weight of *Lucilia cæsar* Linné, from the egg to the adult flesh fly  
(Set No. 2)

1	2	3	4	5	6	7	8	9	10	11
Date, 1905, July	Time	Age in Hours	Weight in Milligrams	Observations	Average in Milligrams	Increase over Last Meas- urement	Interval Be- tween Meas- urements	Average Hourly In- crease	Per- cent In- crease	REMARKS
12	7.30 p.m.	eggs	2	20	.1	.0	—	—	—	eggs
13	10.00 a.m.	3	4	20	.2	.1	2 hrs	.050	50.	larvæ
13	12.00 a.m.	5	2	15	.13	— .07	2 hrs	— .035	— 17.5	
13	2.00 p.m.	7	5	10	.5	.37	2 hrs	.185	142.30	
13	4.00 p.m.	9	5	10	.5	.0	2 hrs	.000	.00	
13	6.00 p.m.	11	8	10	.8	.3	2 hrs	.150	30.	
13	8.00 p.m.	13	10	10	1.	.2	2 hrs	.100	12.5	
13	10.00 p.m.	15	18	10	1.8	.8	2 hrs	.400	40.	
13	12.00 p.m.	17	23	10	2.3	.5	2 hrs	.250	13.88	
14	2.00 a.m.	19	35	10	3.5	1.2	2 hrs	.600	26.09	
14	4.00 a.m.	21	38	10	3.8	.3	2 hrs	.150	4.29	
14	6.00 a.m.	23	52	10	5.2	1.4	2 hrs	.700	18.42	
14	8.00 a.m.	25	65	10	6.5	1.3	2 hrs	.650	12.50	
14	10.00 a.m.	27	82	10	8.2	1.7	2 hrs	.850	13.07	
14	2.00 p.m.	31	173	10	17.3	9.1	4 hrs	2.275	27.74	
14	6.00 p.m.	35	208	10	20.8	3.5	4 hrs	.875	5.06	
14	10.00 p.m.	39	270	10	27.0	6.2	4 hrs	1.550	7.45	
15	2.00 a.m.	43	325	10	32.5	5.5	4 hrs	1.375	5.09	
15	6.00 a.m.	47	330	10	33.0	.5	4 hrs	.125	.388	
15	10.00 a.m.	51	371	10	37.1	4.1	4 hrs	1.025	3.106	
15	4.00 p.m.	57 ±	404	10	40.4	3.3	6 hrs	.550	1.48	migrated
16	10.00 a.m.	75 ±	177	5	35.4	— 5.0	18 hrs	— .278	— .688	
19	7.30 a.m.	145 ± in day:	158	5	31.6	— 3.8	70 hrs	— .054	— .152	pupated
19	7.30 p.m.	6	144	5	28.8	— 2.8	12 hrs	— .233	— .737	
20	7.30 a.m.	7	140	5	28.0	— .8	12 hrs	— .066	— .23	
20	7.30 p.m.	7	133	5	26.6	— 1.4	12 hrs	— .116	— .414	
21	7.30 a.m.	8	132	5	26.4	— .2	12 hrs	— .016	— .06	
21	7.30 p.m.	8	137	5	27.4	+ 1.0	12 hrs	+ .083	+ .315	
22	7.30 a.m.	9	133	5	26.6	— .8	12 hrs	— .066	— .24	
22	7.30 p.m.	9	133	5	26.6	.0	12 hrs	.000	.00	
23	7.30 a.m.	10	133	5	26.6	.0	12 hrs	.000	.00	
23	7.30 p.m.	10	133	5	26.6	.0	12 hrs	.000	.00	
24	7.30 a.m.	11	133	5	26.6	.0	12 hrs	.000	.00	
24	7.30 p.m.	11	133	5	26.6	.0	12 hrs	.000	.00	
25	7.30 a.m.	12	132	5	26.4	— .2	12 hrs	— .016	— .06	
25	7.30 p.m.	12	128	5	25.6	— .8	12 hrs	— .066	— .25	
26	7.30 a.m.	13	128	5	25.6	.0	12 hrs	.000	.00	
27	7.30 a.m.	14	124	5	24.8	— .8	24 hrs	— .066	— .25	
28	a.m.	15	115	5	23.0	— 1.8	24 hrs	—	—	imagines

\* Prepared in part after Minot ('91).

One set of *Sarcophaga* was weighed at two-hour intervals and three sets at four, six and eight-hour intervals, respectively. After the individuals were well in the pupal stage all weighing was carried on every twelve hours until the imagines emerged.

TABLE III

Showing the average changes in weight of *Sarcophaga sarraceniæ* Riley, from the larva at extrusion to the adult flesh fly (Set No. 8)

1	2	3	4	5	6	7	8	9	10	11
Date, 1905, July	Time	Age in Hours	Weight in Milligrams	Observations	Average in Milligrams	Increase over Last Meas- urement	Interval Be- tween Meas- urements	Average Hourly In- crease	Hourly Per- cent In- crease	REMARKS
13	10.30 a.m.	1	4	20	.2	.0	—	—	—	migrated pupated
13	1.30 p.m.	3	4	15	.26	.06	3 hrs	.020	10.	
13	7.30 p.m.	9	11	10	1.1	.84	6 hrs	.140	53.84	
14	1.30 a.m.	15	22	10	2.2	1.1	6 hrs	.183	16.63	
14	7.30 a.m.	21	53	10	5.3	3.1	6 hrs	.516	23.45	
14	1.30 p.m.	27	112	10	11.2	5.9	6 hrs	.983	18.54	
14	7.30 p.m.	33	185	10	18.5	7.3	6 hrs	1.216	10.86	
15	1.30 a.m.	39	222	10	22.2	3.7	6 hrs	.616	3.33	
15	7.30 a.m.	45	298	10	29.8	7.6	6 hrs	1.266	5.703	
15	1.30 p.m.	51	300	10	30.0	.2	6 hrs	.033	.111	
15	7.30 p.m.	57	246	5	49.2	19.2	6 hrs	3.200	10.67	
16	9.30 a.m.	71 ±	451	5	90.2	41.0	14 hrs	2.928	5.95	
19	7.30 a.m.	141 ± in days	459	10	45.9	44.3	70 hrs	-.632	-.701	
19	7.30 p.m.	6	439	10	43.9	-2.0	12 hrs	-.166	-.362	
20	7.30 a.m.	7	432	10	43.2	-.7	12 hrs	-.058	-.132	
20	7.30 p.m.	7	427	10	42.7	-.5	12 hrs	-.041	-.095	
21	7.30 a.m.	8	423	10	42.3	-.4	12 hrs	-.033	-.077	
21	7.30 p.m.	8	420	10	42.0	-.3	12 hrs	-.025	-.059	
22	7.30 a.m.	9	420	10	42.0	.0	12 hrs	.000	.000	
22	7.30 p.m.	9	414	10	41.4	-.6	12 hrs	-.050	-.12	
23	7.30 a.m.	10	411	10	41.1	-.3	12 hrs	-.025	-.06	
23	7.30 p.m.	10	409	10	40.9	-.2	12 hrs	-.016	-.039	
24	7.30 a.m.	11	411	10	41.1	+.2	12 hrs	+.016	+.039	
24	7.30 p.m.	11	407	10	40.7	-.4	12 hrs	-.033	-.08	
25	7.30 a.m.	12	404	10	40.4	-.3	12 hrs	-.025	-.061	
25	7.30 p.m.	12	403	10	40.3	-.1	12 hrs	-.008	-.02	
26	7.30 a.m.	13	401	10	40.1	-.2	12 hrs	-.016	-.039	
27	7.30 a.m.	14	399	10	39.9	-.2	24 hrs	-.008	-.02	
28	7.30 a.m.	15	399	10	39.9	.0	24 hrs	.000	.00	
30	7.30 a.m.	17	393	10	39.3	-.6	48 hrs	-.012	-.03	
31	a.m.	18	355	10	35.5	-3.8	24 hrs	—	—	imagine

*Sarcophaga sarraceniae* endured the experimental conditions far better than *Lucilia* as was evident from the number of imagines resulting in each case—90 per cent in the former and only  $16\frac{2}{3}$  per cent in the latter, including the set which perished entirely. The greatest mortality occurred during the period between migration and pupation, though a good portion never completed the period of pupation after having entered it—about 40 per cent in *Lucilia* and about 6 per cent in *Sarcophaga*.

In order to secure a fair weight it was necessary to brush each larva clean with a camel's hair brush. This great amount of handling had practically no effect on *Sarcophaga*, which are quite hardy, but on the other hand *Lucilia* were seriously affected.<sup>6</sup> However, in the latter case it is well worth noting that the survivors are very near the mode of normal frequency for larvæ at migration, for pupæ and for imagines (*cf.* normal frequency curves, Fig. 7, with growth curve, Fig. 2).

This growth as indicated by the curves was checked by the growth of larvæ, both in time and weight, feeding on fish out of doors under normal conditions, and it was found that the time is the same, and that the average weight of migrating larvæ varied but 0.5 mg. This difference would, undoubtedly, have been entirely wiped out had a larger number of individuals been used.

#### *Discussion of Curves*

*Lucilia cæsar* (Fig. 2): The figure shows a curve derived from a set weighed every two hours for the first twenty-four hours after hatching, every four hours during the following twenty-four, every six hours for the next twelve, etc., as indicated in Table II, column 2, according to which this curve is constructed. The curve begins with the weight of ten eggs (1 mg.) This egg stage is represented by the dotted line and covers a period of about twelve hours. At this point the larvæ hatched and were weighed three hours after the emergence of the first individual with a weight of 4 mg., for twenty or 2 mg. for ten, as shown by the curve. The average increase in weight during the first three hours is 50 per cent which

<sup>6</sup>This may not have been entirely due to the handling; the same mortality for this species may possibly exist in natural conditions.

is followed during the next two hours by an average decrease of 17.5 per cent. This decrease was shared by three sets, and two further sets showed zero growth, which was probably due to the fact that the weighing was done a half to three-quarters hour later, during which time the larvæ gained enough to bring the process from negative to zero. Neither does the sixth set show the decrease because it was weighed still later. Five of the six sets of *Lucilia* were thus affected, as is shown by Table I.<sup>7</sup>

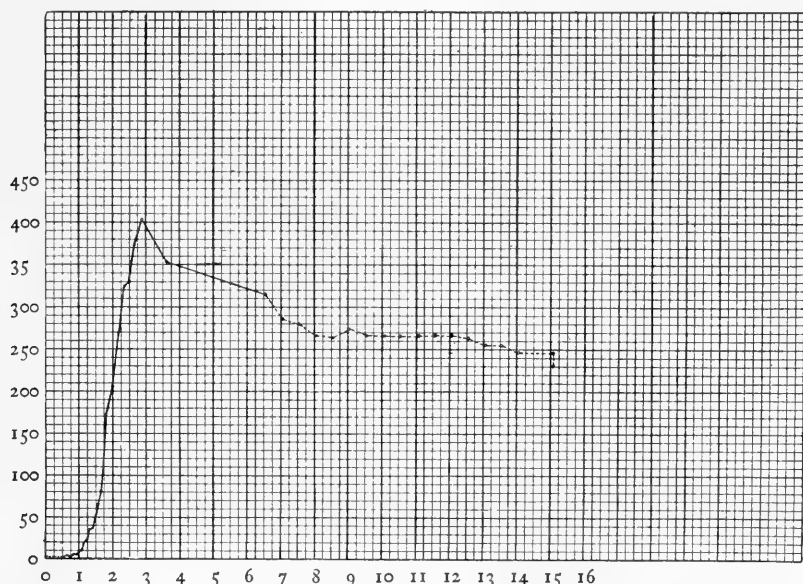


Fig. 2 Growth curve of *Lucilia cæsar* Linné, derived from Table II, column 4, on the basis of 10 individuals;  $x$  = days;  $y$  = milligrams. The dotted line at the beginning of the curve represents the egg stage; the solid line represents the larval period with the apex as the point of migration; the broken line represents the period of pupation and the drop at the end, the loss of the pupa cases.

Migration is represented by the apex of the curve when the larvæ reach an average weight of 40.4 mg. and this for a feeding period of about sixty hours (57+ hours), an average hourly increase of .7070 mg. or an increase of 40,400 per cent. of the original weight.

Immediately after migration there is a marked loss in weight.

<sup>7</sup>Loss of weight probably due to loss of moisture in crawling on dry surface of receptacle.

Since there is no appreciable fæcal discharge and because the loss is quite gradual, it is assumed to be due to loss of moisture. This loss is less pronounced after the fourth day until the seventh day when pupation takes place. The pupal period is marked by the broken line. Here there is an abrupt loss in weight. During this early period of transformation from larva to pupa the case changes in color from a pale yellow to the characteristic chestnut of the advanced pupa. This change in color, due to the hardening of the chitin, is quite rapid. In the eighth day there is another drop

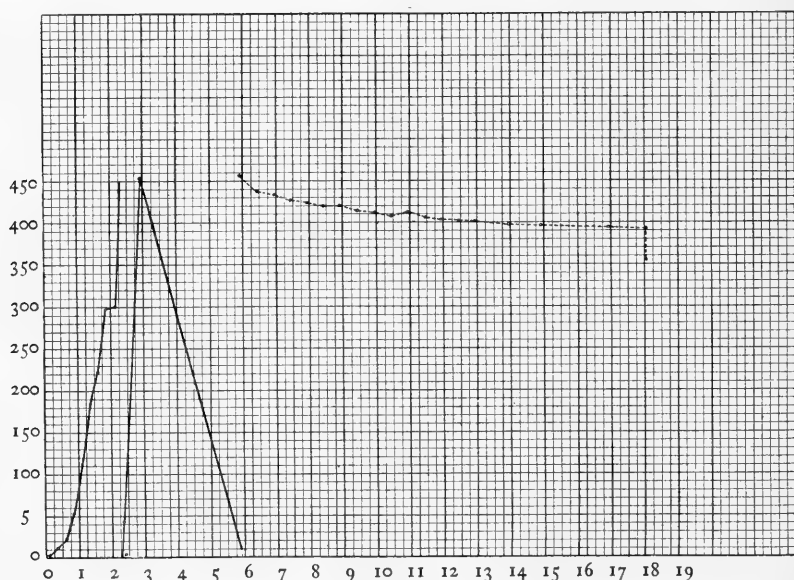


Fig. 3 Growth curve of *Sarcophaga sarraceniarum* Riley, derived from Table III, column 4, based on 10 individuals;  $x$  = days;  $y$  = milligrams. The solid line represents the larval period with apex as the point of migration; the broken line represents the pupal period and the drop at the end, the loss of the pupa cases.

which is followed in the ninth day by a considerable increase. This is also evident in the remaining sets weighed at about this same age. This increase of weight shared by practically all pupæ weighed, is probably due to an addition of moisture extracted from the air, or there may be a correlation to internal metabolism. At this point in the life history the metamorphosis is wonderfully rapid. During the first day or two of pupation the individual

seems to disintegrate, forming a mass of fluid matter, but in the third day there is a rapid organization, which results in an individual that could be easily recognized as a fly, even at this early period.

All pupæ were kept in glass vials about two-thirds full of sand in which they were buried. These vials were covered with netting (bobbinet), and were kept near a window which was kept open most of the time together with other windows in the room. That the sand, which was comparatively dry, might extract moisture from the air and in turn transmit it to the pupæ is very probable.

The increase in weight above mentioned is followed by a nearly corresponding decrease during the next twelve hours, which resulting weight is held for two days and a half. At the end of this time the weight again gradually decreases until during the last day of pupation when it is uniform. The abrupt drop at the end of the curve represents the casting off of the pupa cases.

*Sarcophaga sarracenizæ* (Fig. 3): This curve is based on the growth of a series weighed every six hours during the feeding period (see Table III, column 2). The curve shows several interesting features. In the first place the increase in weight is really prodigious, beginning with an average weight of 0.2 mg. (10 larvæ equals 2 mg.) and increasing to an average of 90.2 mg. in seventy-one hours, an average hourly increase of 1.270 mg. or an increase of 45,100 per cent of the original weight. This average for larvæ of this species is not high since many individuals which were weighed for another purpose ranged from 150 to 200 mg. (75,000 to 100,000 per cent) and over and were probably developed from larvæ weighing no more than the above at extrusion. This species did not show a decrease in weight at the beginning of the feeding period as *Lucilia*.

Another remarkable feature is the large and rapid drop in weight after migration. This loss is just about one-half of the average original weight of the migrating larvæ. The pupal period is characterized by a small but comparatively regular loss in weight.

Percentage increment curves (Figs. 4 and 5): Since applying the percentage increment method used by Minot ('91) the writer

is convinced that this is the true method of growth measurement and curves constructed on this basis show the facts in a remarkably clear manner.

It will be seen at a glance that *the tendency is for growth to decrease in rate uniformly*, and that there is a wave-like rise and fall

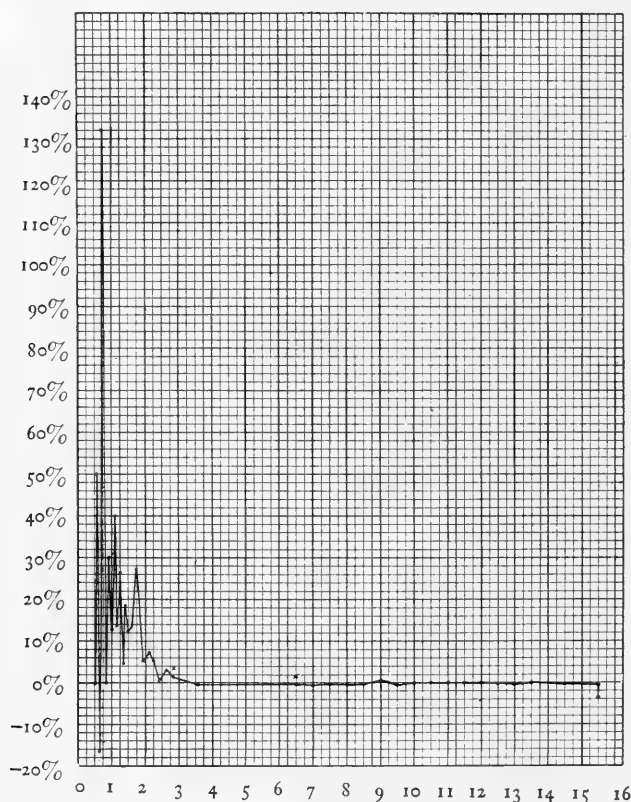


Fig. 4 Growth curve of *Lucilia caesar* Linné, based on percentage increments. Derived from Table II, column 10;  $x$  = days;  $y$  = percentage increments. Crosses represent point of migration and point of pupation.

with a deep trough and a high crest at the beginning of growth which gradually diminish with age. It is apparent that the conclusions reached by Minot ('91) also apply here, viz: "The study of the individual variations yields two important conclusions: First, That any irregularity in the growth of an individual

tends to be followed by an opposite compensating irregularity. Second, The variability diminishes with the age," also, "the irregularity of growth of an individual is very great" and "each individual strives to reach a particular size." As a proof for this latter statement, see Table I. The average weight of migrating *Lucilia* larvæ for the five sets shown is, viz: 38.6, 40.4, 39.4, 36.0 and 39.0 mgs.

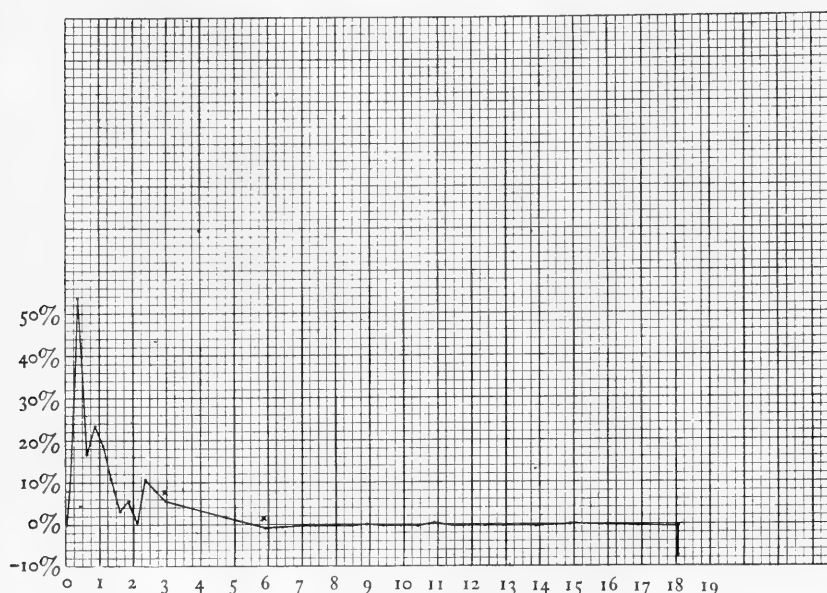


Fig. 5 Growth curve of *Sarcophaga sarracenizæ* Riley, based on percentage increments, derived from Table III, column 10;  $x$  = days;  $y$  = percentage increments. Crosses represent point of migration and point of pupation.

#### IV CORRELATION OF FEEDING PERIOD WITH FOOD SUPPLY

That there exists a correlation between the feeding period of the larvæ and the food supply, there can be no doubt. My attention was called to this fact time and again. To illustrate, one instance follows: On July 28, 1905, a fish was exposed for fly eggs and about one thousand were secured between 1.45 and 3.45 o'clock, p. m., not a large number. The eggs were distributed over several pieces of the flesh, the larvæ emerging early July 29. As early as noon of July 31 (54 hours feeding), the larvæ on the



smallest piece had eaten it clean and had migrated, while almost all larvæ on the larger pieces continued feeding until about noon of August 1 (67 to 72 hours feeding) when practically all migrated. A few, however, continued feeding on the unconsumed flesh and the last larva did not migrate until after noon of August 3.

That the larvæ on the last two pieces of fish did not continue eating until the flesh was consumed, indicates that there is an *optimum* when enough nourishment has been taken to pass through the metamorphosis to best advantage, and at this point migration takes place. That this optimum is not always reached is shown by the early migration because of food shortage, and this does occur at times out of doors, since eggs and larvæ may be deposited in such large numbers on a single fish that an early migration is necessary. Thus we have sharp competition which is further augmented by the pressure of four species, each more voracious than the other if that were possible. There has been, undoubtedly, a gradual adaptation in the past to the existing conditions. In order that the flies might exist in the locality, there must have been a conformation to the food supply, *i. e.*, a race of flies which can adapt itself to an inconstant, somewhat periodic supply of food would survive. Further there must have been an adaptation of the four species already mentioned to each other, a species which required a comparatively long feeding period could not well exist with a species whose feeding period was of short duration. The food would invariably be consumed by the quick feeder, while the former would suffer starvation. As it is, all larvæ migrate at about the same time, *i. e.*, when the fish is consumed which usually requires a fairly uniform time, as above noted. A glance at the larvæ at this time will reveal the fact that there are two general sizes of larvæ present, very large ones and small ones. All have fed during approximately the same time, yet *Sarcophaga sarra-cenæ* has attained its enormous size while *Lucilia cæsar* is uniformly smaller. The greatest variation in size is found in the former. The larvæ of the screw-worm fly are usually smaller in average than *Lucilia cæsar*.

There is probably also a correlation with the surf producing storms, which is discussed in full later in this paper. Another

factor which requires a hasty consumption of the flesh is the drying out caused by the sun, and also the decay from putrefaction.

The above general observations led to the following described systematic experiments bearing on the subject of correlation with food supply.

#### V EFFECTS OF OVER AND UNDERFEEDING

The observations described in the last chapter were interpreted to mean three things, viz: First, That there exists an *optimum* at which a certain larval weight is reached, which is the weight best adapted to pupation and emergence as imagines. Second, That if feeding is carried on *beyond* this optimum point, pupation is hindered or even death may ensue. Third, There must be a point *below* the optimum at which the larvæ can barely pupate, and have not strength enough to carry through the pupal period or will even die before pupation. To determine the facts and to find the critical point between death or survival, the series of experiments about to be described was arranged.

On August 12, 1905, a large German carp, quite fresh, was washed up. This carcass was immediately taken from the beach, placed near the laboratory and exposed for eggs of *Lucilia cæsar*. In an hour and a half (between 10 and 11.30 o'clock, a. m.), about eleven thousand eggs were deposited, the gross weight of which was 1083 mg. The fish was taken into the laboratory, the viscera removed, the body cut into six pieces, and the eggs roughly divided into six masses. These masses were then placed upon the pieces, which in turn were placed in separate boxes or compartments. Two of these sets were used for histological material, and consequently only four series (series 1, 2, 3, 4) were used in the experiment. The eggs hatched early August 13. The plan was to take the flesh away from a portion of each set at consecutive intervals of six hours each, allowing one series to migrate normally, weighing the larvæ at end of feeding period. Then the weighed larvæ were placed in separate dishes so that the same lot could be weighed again about the middle of the period of pupation, and the adults when the wings and bodies were dry. The remainder of each series was retained as a check or in case of accident.

This first experiment was supplemented by a second one a little later (August 28) and carried out on exactly the same plan (series 5, 6). To show the results of this series of experiments more clearly the following table is presented:

TABLE IV

*Showing results of Feeding Experiments on Lucilia caesar, the common green flesh fly*

Series No.	Feeding Period in Hours	Mean Weight of Larvæ at End of this Period	Mean Weight of Pupæ	Mean Weight of Imagines
(1) optimum	60-72	38.183(256)	30.283(289)	22.283(208)
2	60	35.68 (50)	24.76 (50)	18.44 (47)
3	54	31.06 (50)	22.38 (48)	17.54 (48)
4	48	22.14 (50)	11.81 (48)	8.08 (44)
5	42	17.06 (50)	(39)	12.38 (31)
6	36	8.82 (57)	9.34 (49)	7.15 (20)
7	retarded	33.59 (64)	(39)	21.00 (25)
8	60-78	46.73 (65)	dead larvæ	—
9	55	36.78 (50)	larvæ having migrated early	—

Note 1—Weight was carefully taken in milligrams.

Note 2—Figures in ( ) denote number of individuals weighed.

Note 3—The same individuals were weighed in each series except in cases mentioned below.

Note 4—See Fig. 6 for relative sizes of imagines.

## EXPLANATION OF TABLE IV

*Series 1*—The term optimum as here used represents larvæ in greatest frequency and also corresponds to the normal frequency of the group which was shown by the weight taken of individuals feeding out of doors, as stated above. This series accordingly forms a convenient basis for comparison in reference to the remaining series. The normal frequency curves (larvæ, pupæ and imagines) of *Lucilia caesar* are based on this series. The feeding period here indicates that the larvæ migrated after eating for from 60 to 72 hours. This optimum weight for larvæ (mean 38.183 mg. and mode 37.00 mg.) represents the point at which the chances are best for pupation and emergence as adults. From this point either way the chances diminish, most rapidly, of course, at the extremes. The pupa cases of optimum forms and

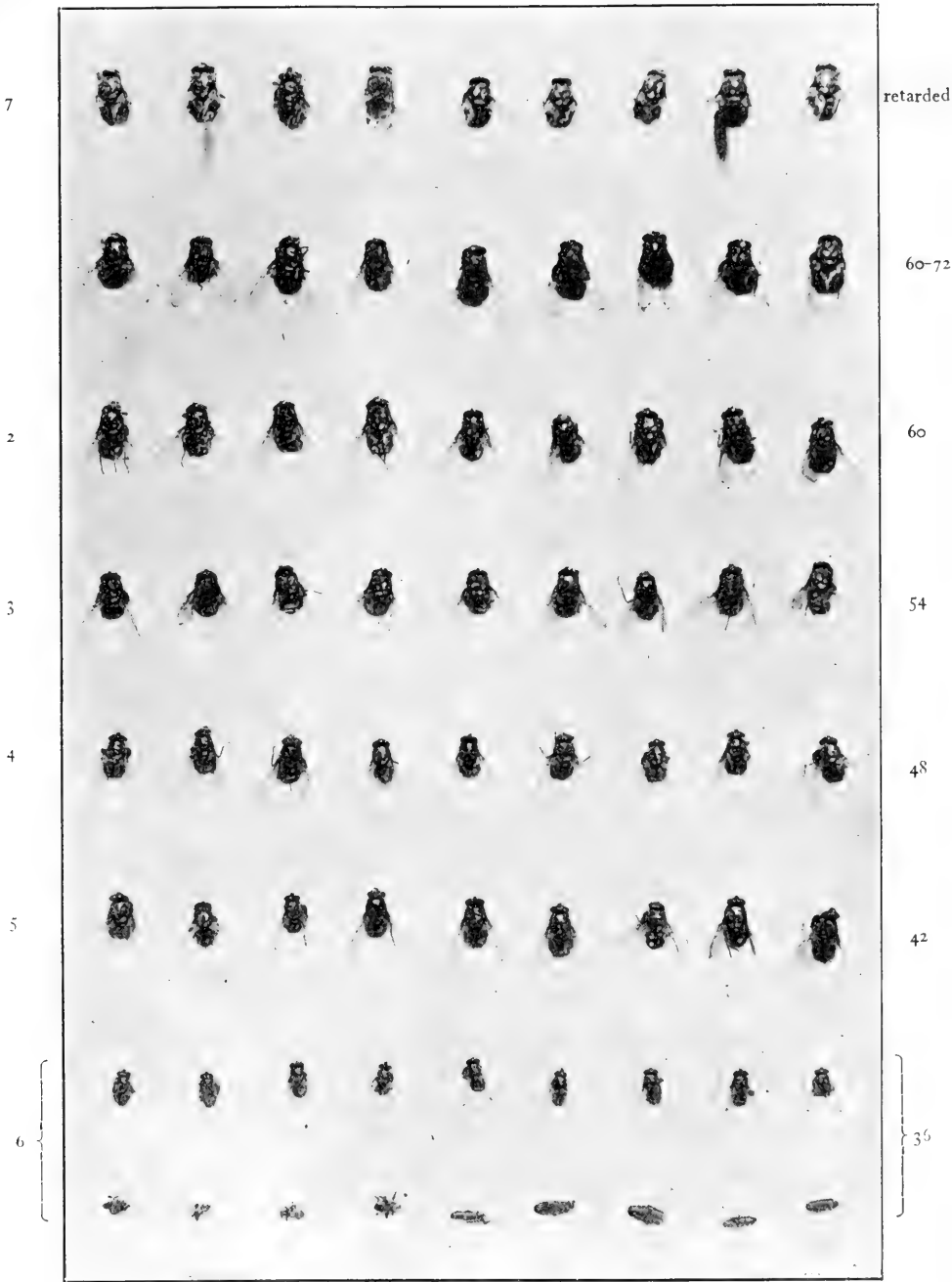


Fig. 6 Cut showing relative sizes of imagines as produced by feeding experiments. Number of the series on the left, and number of hours that the larvæ were permitted to feed on the right. See explanation of series on pp. 68 to 72.

beyond are very chitinous, making a comparatively rigid shell, which affords the optimum of protection. On the other hand, the farther below the optimum, the less rigid the case, until at the lowest extreme it is a mere flimsy covering. This shows that here the least possible energy is expended, while the greatest amount possible is stored up for the trying transformation from larva to imago.

*Series 7*—Among the optimum series there were certain larvæ which were very slow in pupating. While others of the same series pupated August 18 to 23, these particular ones had not by August 27, the date on which they were weighed to ascertain the reason for such tardiness. This weighing, in which 64 larvæ were taken, showed a mean of 33.59 mg., which in itself does not show a high average (*cf.* series 1, larvæ). If we consider the lapse of time since migration (twelve days) and the loss of moisture undergone during this time, we must see that this average is after all high, and that these larvæ were beyond the optimum weight, and for this reason pupation was deferred. (For loss of moisture *cf.* curve of growth for *Lucilia cæsar*.) Of these 64 larvæ 39 pupated by September 9, giving us twenty days in the larval period against a normal of about six days; the rest were dead. Furthermore, at the present writing only 25 adults have emerged out of the 39 pupæ. The mean of these adults is 21 mg., which shows pointedly that the heaviest larvæ had been eliminated or had dried out sufficiently, resulting in adults very near the optimum weight (*cf.* adults in series 1).

*Series 8*—The sixty-five larvæ in this series were picked from the same fish upon which the optimum series was feeding, and consequently could not have been poisoned. All the dead larvæ without exception were taken and weighed so that no selection was possible. The larvæ were carefully examined for injuries, but only one was found to show an injury, the smallest of the lot (25 mg.) In this case it was decided that death was due to this cause and the weight was not included in the result shown.

Thus we have what was expected, *i. e.*, that continued feeding without regard to the optimum would eventually result in death. The mean for this series 46.73 mg. is extremely high and a com-

parison with the normal frequency curve for larvæ shows that the aberrant forms beyond the optimum shown in the curve are either just within the above mean or just beyond. It is also interesting to know that 63 per cent of the total number of dead larvæ are beyond 45 mg. in weight.

*Series 9*—It will be seen that these larvæ *migrated* five hours sooner than other migrating individuals, but that the weight on the other hand is nearly equal to the optimum. This corroborates the statement made above that some individuals eat more rapidly than others, and again this series augments the evidence in favor of an optimum weight.

*Series 2*—In this series the optimum time limit for feeding is practically reached, but, since these larvæ were still feeding at sixty hours, the flesh was taken away, and we see that the optimum weight had not yet been reached by several milligrams. This indicates again, when compared with series 9, that certain larvæ feed faster and reach the optimum more quickly than others.

*Series 3*—Food taken away after feeding fifty-four hours. Pupation takes place readily and promptly and adults emerge on time, but are short weight and small in size.

*Series 4*—Food taken away after feeding forty-eight hours. Pupation takes place as above, also same for imagines. For those series below the optimum down to and inclusive of forty-two hours, the average time between migration, or in these cases time of taking food away, and pupation, is very much more regular and nearer the normal time, *i. e.*, about four days. This might be expected, since in the normal series we have many larvæ which have gone beyond the optimum weight and pupation is consequently retarded. On the other hand, in the series just mentioned the larvæ are practically all within the optimum, consequently the time for the entire life history of each individual conforms more closely to the normal and there is no dragging out of the prepupal period.

*Series 5*—In this series from which the food was taken away at forty-two hours, all the original weighed larvæ died because of an accident. As a result the pupal weight is not given, but the weight of the adults was taken from the accessory series. The number of individual larvæ in the accessory series was not taken, but only thirty-nine pupæ resulted, and from these only thirty-

one adults emerged, whence the number used in this series. The mean weight shows a remarkable selection, since the average weight of the adults is greater in this series than in the preceding series, and yet the mean weight of larvæ is less, that is to say, the lighter weight larvæ did not produce adults.

*Series 6*—In this series with a feeding period of thirty-six hours we reach the lower limit at which adults could be secured. *The smallest fully developed flies of Lucilia cæsar weighed about four milligrams*, though several emerged that weighed but half that much, but died before the wings were spread. Several also died in struggling to free themselves from the pupa cases. As mentioned before the pupa cases of these light forms are very flimsy and are also gummy, thus making it more difficult for the young imagines to emerge from such a case.

Note 1—The weights given above for imagines are of flies that had no opportunity of feeding, the weight being taken within an hour or two after emerging from the cases. For comparison the individual weight was taken of sixty-four specimens of *Lucilia cæsar*, regardless of sex, feeding out of doors on fish. The range of weight was quite wide, varying from 14 mg. to 72 mg., however, only three were above 60 mg. The average weight of this group is 38.17 mg. As compared with the adults of the optimum series (22.28) the above average seems very great. This can be accounted for by the presence of numerous ova in various degrees of development within the females and also by the presence of liquid food that is contained within the alimentary canal of each individual.

Note 2—It was the plan of the writer to secure the normal frequencies of *Sarcophaga sarraçeniæ*, but the great variation in weight required more individuals than there was time for weighing. After weighing several hundred larvæ at migration, the result showed a range of weight from 75 mg. to 227 mg., and in no class were there more than eight variates. No less than five thousand larvæ would be necessary to establish the normal frequency of this species. This wide variation in larvæ at migration means also a wide variation in pupæ and imagines, which was also partly worked out.

Tables showing the distribution of frequencies in *Lucilia cæsar*:

TABLE V

*Larvæ at migration*

Classes..	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57
Variates	1	1	1	0	3	6	4	7	7	13	18	25	30	24	23	22	16	15	15	11	5	2	2	2	1	0	0	1	0	0	0	0	1

TABLE VI

*Pupæ weighed about the middle of the pupal period*

Classes.....	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46
Variates .....	1	1	2	2	6	9	13	16	18	19	31	20	20	19	20	17	16	9	16	10	6	7	5	0	4	1	0	0	1

TABLE VII

*Imagines weighed two or three hours after the wings were spread; no food taken*

Classes.....	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
Variates .....	2	0	3	4	11	10	16	22	23	23	21	21	16	11	8	7	3	1	1	2	1	1	0	1

TABLE VIII

Constants based on Tables V, VI, VII, and derived by the usual formulae:

$$\left[ A = \frac{\Sigma(V \cdot f)}{n}, E_A = \pm 0.6745 \frac{\sigma}{\sqrt{n}}; \sigma = \sqrt{\frac{\Sigma(x^2 \cdot f)}{n}}, E_\sigma = \pm 0.6745 \frac{\sigma}{\sqrt{2n}}; C = \frac{\sigma}{A} \times 100\%, E_C = \pm 0.6745 \frac{C}{\sqrt{2n}} \left[ 1 + 2 \left( \frac{C}{100} \right)^2 \right]^{\frac{1}{2}} \right]$$

	Larvæ	Pupæ	Imagines
$n$ (Variates).....	256	289	208
$A$ (Average) .....	38.183 ± .180	30.283 ± .197	22.283 ± .176
$M$ (Mode) .....	37.	28.	21. or 22. (?)*
$\sigma$ (Index of Variab.).....	4.293 ± .127	4.974 ± .139	3.769 ± .124
$C$ (Coeff. of Variab.).....	11.24 ± .33	16.42 ± .47	16.91 ± .56

\*See Table VII.

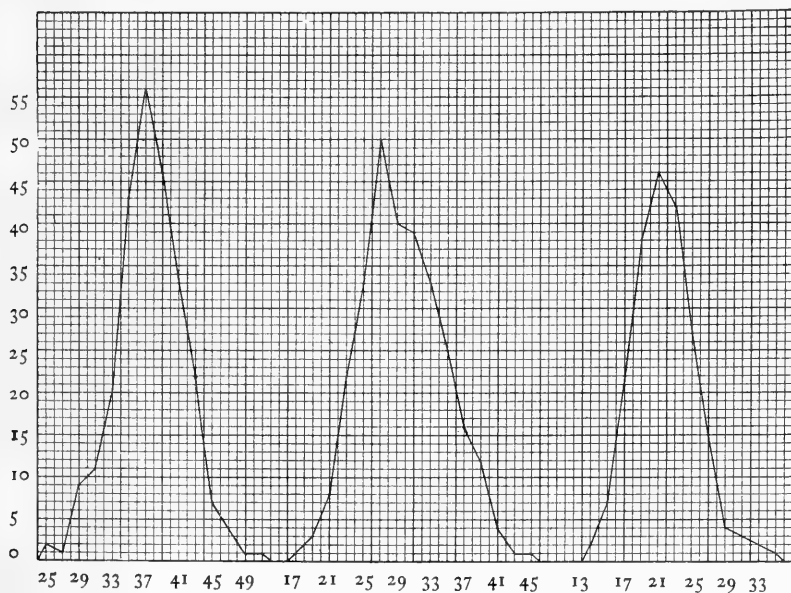


Fig. 7 Normal Frequency curves of *Lucilia cæsar* Linné, based on Tables V, VI and VII, respectively. The classes based on weight in milligrams are shown along the abscissa and the number of variates along the ordinate. The first curve, based on 256 individuals, is of larvæ at migration; the second curve, based on 289 individuals, is of pupæ at about mid-pupal period; the third curve, based on 208 individuals, is of imagines shortly after emergence.

Note—It is practically impossible to weigh all pupæ at the same stage, which accounts for the irregularity of the derived curve. The weight of the imagines was taken several hours (2 to 3) after emerging from the pupa cases. It should be borne in mind that these imagines had not taken food before weighing. This seems to be the best plan for the derivation of a curve with a reasonably uniform basis.



# VI CORRELATION OF LIFE HISTORIES TO THE SURF-PRODUCING STORMS

As already intimated the surf-producing storms occur at comparatively regular intervals, a low surf taking place about every three days, a heavier surf every six or seven days, and a still heavier surf every fourteen or fifteen days. Now the very fact that the life histories of *Comptosmyia macellaria*, *Sarcophaga assidua*, *Lucilia cæsar*, and *Sarcophaga sarracenix* cover, respectively, a period of eight or nine days, twelve or thirteen days, fourteen or fifteen days, and eighteen or nineteen days, seems to indicate a peculiar coincidence, if nothing more. The factor 3 plays an important rôle, viz:  $3 \times 3$ ,  $3 \times 4$ ,  $3 \times 5$  and  $3 \times 6$ , which corresponds in general to the occurrence of the surf. Does this signify anything, or is it merely coincidence?

In an endeavor to interpret this and to have a working basis, a table was secured of the northeast and east winds (*i. e.*, the surf-producing winds on Cedar Point) prevailing at Sandusky during May, June, July and August for the years 1901 to 1905, inclusive. This table was furnished through the kindness of Mr. E. H. Nimmo, Director of the U. S. Weather Bureau Station at Sandusky, Ohio. This table confirms in general the observations made above. In it are given the dates on which the prevailing winds were from the direction favorable for a surf. For the four months named above in the years 1904 and 1905 are found the following intervals between storms in days:

1904—3, 14, 7, 6, 3, 6, 3, 9, 12, 6, 6, 9, 6, 6, 3, 6.

1905—9, 12, 4, 3, 3, 3, 2, 8, 4, 6, 3, 6, 6, 6, 9, 6, 6, 3, 9.

It can readily be seen that 3 is again the prominent factor. Fish are only cast up in quantities by a surf, and a surf is alone caused by a prevailing wind from the northeast or east. These fish are practically the sole food for the flies along the beach and this is especially true of those individuals living on the narrow strip of sand called Cedar Point, upon which the laboratory is situated. Were adults to emerge from their pupa cases at a time when no fish or very few fish were present on the beach the proba-

bilities are that such individuals would suffer starvation. If this were often repeated the tendency would be to impair the vigor of the species, especially by interfering with the normal egg-laying habit. This latter would certainly be the case if the usual number of adults were to emerge with an undersupply of food present upon which the eggs could be deposited. The large number of larvæ for the short supply of food would result in producing smaller individuals, which has been proven by experiments. That the sarcophagids given in the list are normal, as compared with individuals of the same species breeding elsewhere, is evident to the most superficial observer, and they are certainly not less numerous.

Considering the above facts and also bearing in mind that the food supply is influenced by the comparative regularity of the surfs, there seems then to have been somewhere in the past an adaptation to the surf-producing storms. When the adult fly emerges from the pupa case it is likely to find available food on the beach, or has but a very short time to wait for it. Then since egg deposition and food supply are so intimately connected, eggs are deposited and the cycle begins anew.

As soon as the liquids have been sucked from the accessible parts of the fish, egg-laying ceases and the remainder of the work is left to the larvæ. The presence of juices would then seem to be a gauge for regulating the number of eggs and young larvæ deposited on one fish by the females. This will recall the statement made above that eggs are seldom if ever deposited on fish that have become dry, which fact should also be borne in mind in connection with the adaptation to the surfs. If fish were to lie around for any length of time before the flies emerge, the juices would be dried up by the sun, and the fish would become unfit for food. However, it is very probable that the adults would after all deposit eggs on the dry fish. Lack of food for the adults would necessarily be a serious menace to the species. Under conditions as they now exist a drying out of a fish by the sun would not likely occur, since the flies would not permit a single fish to dry out thus. The assertions relating to this are based on laboratory experiments, *i. e.*, drying out a fish in the laboratory and then placing it outside in reach of flies.

That which is of principal interest in regard to this correlation of life histories to the surf-producing storms is the brief interval between the storms, represented by the factor 3 or 6, and this with its relation to the days required for development with each species, viz: *Comptosmyia* about 9 days, *Sarcophaga assidua* about 12 days, *Lucilia* about 15 days, and *Sarcophaga sarraçeniæ* about 18 days. Further, it must be remembered that the life history of each species for this locality covers a comparatively definite period, which is a necessary consideration in this matter of correlation. When eggs or larvæ were collected, very little chance was involved in predicting the date on which the imagines would appear. The writer made use of this factor in his experiments with the three most abundant species. It would also be useless to speak of a correlation to the surf producing storms if the life histories of the species studied here corresponded to the life histories of the same species in localities remote from a beach. From the literature consulted the following data was secured relative to the latter.

*Comptosmyia macellaria*: Morgan ('90) gives (August 18 to August 29-30) *11 to 12 days*; Francis ('90) larval stage about a week and pupal stage from 9 to 14 days, a total of from 16 to 21 days.

*Sarcophaga assidua*: Howard ('00) gives (July 3 to July 25) *22 days*, also (July 9 to July 18-26) *9 to 17 days*.

*Lucilia cæsar*: Howard ('00) gives (May 12 to 29) *17 days*.

*Sarcophaga sarraçeniæ*: Howard ('00) gives (May 12-30) *18 days*; (July 2-29) *27 days*; (June 6-17) *11 days*; (June 13-26) *13 days*; (July 7-21) *14 days*; (July 9-22) *13 days*; (July 24 to August 9-11) *16 to 18 days*; Kellogg ('05) gives *10 to 12 days*, Howard ('02) *10 days*.

One can readily see from the above citations that there is a marked variation in each species, and that these periods do not coincide very closely (excepting the first period in the last-named species) with the results secured in these studies. It must, however, be admitted that more extensive and systematic work should be done relating to the question under discussion.

Conditions as stated above may lead to the impression that egg deposition is a direct result of the presence of food within the

alimentary canal. But this is evidently not a necessity, as is shown by the following observations.

In carrying on experiments it was always a matter of concern from the first to guard against outside larvæ. On several occasions fish were covered by a screen of netting to keep out flies, but the females of *Sarcophaga sarracenix* invariably deposited their young on the netting and these then found the fish without much difficulty. While carrying on experiments indoors the flesh was kept in Petri dishes and covered with like dishes. Several times it happened that a female of *Sarcophaga sarracenix* gained entrance to the room through the door and deposited larvæ on the outside of the dishes. Not being able to get at the flesh the larvæ perished. Furthermore, in such cases where the head of the fish was hooded with cloth, the females of *Lucilia cæsar* deposited eggs very freely on the cloth and also on the loose ends of the string used to tie the hood. These observations led the writer to believe that it is not necessarily the presence of food *within* the alimentary canal that stimulates egg deposition. In this connection, however, it might be interesting to note, that no eggs were secured from individuals of *Lucilia cæsar* kept under confinement with plenty of accessible food. The flies crawled about on the fish apparently sucking the juices, but all died in a short time. Confinement very probably was the cause. This evidently agrees with experiments on the house fly cited by Howard ('00), viz: "I am inclined to believe that what may be termed the psychological influence of confinement, even in so large an enclosure as the one used in the 1898 experiments, alarmed the flies, caused their early death, and prevented them from obeying their natural instincts and performing their natural functions."

#### VII EXPERIMENTS UPON THE TROPISMS OF FLY LARVAE

The following experiments and observations are not intended to cover the topic of tropisms and their relation to fly larvæ with any degree of thoroughness. The object of this final chapter to the general paper is to present a statement of experiments made on movements in reaching the food and in migration, including a preliminary discussion.

*Chemotaxis*

Two experiments were tried with reference to chemotaxis.

*First Experiment*—Thirty-six larvæ between four and five hours old, were placed in a small vial 4.3 cm. in length, and a piece of fish weighing about one gram was placed 1.5 cm. from the bottom of another vial 12.7 cm. in length. While this was being done, care was taken that the flesh did not come in contact with the sides of the long vial. After the larvæ were shaken to the bottom of the smaller vial, the two were put mouth to mouth horizontally on a table. This took place at 9.05 o'clock, a. m., June 30, 1905. In three or four minutes there was a decided movement toward the mouth, but because of the unevenness of this region, the movements became scattered. At this juncture a short piece of paper was placed like a bridge inside the vials connecting them.

The following table shows the results of the experiment. Unfortunately the data taken were not sufficient to make a complete table.

*Second Experiment*—A small piece of fish weighing about two grams was placed in the center of a large sheet of heavy white paper, then young larvæ were put at different distances from the flesh, after dipping them partly (posteriors) in glycerine so that a trail would be left in crawling.

The *first* larva was placed with head toward the meat at 9 cm. distance, and reached the food in *four minutes* after taking a somewhat winding course. The wind was favorable in this case.

A *second* larva was placed at a distance of 11 cm. with its head away from the flesh and the wind at right angles. This larva started at 10.32 o'clock, a. m. and after a very circuitous route, circling frequently though always drawing nearer and never going beyond, reached the food at 10.52 o'clock. Time, *twenty minutes*. The course of this larva took it considerably to one side of the flesh almost to the starting place of the first larva from which point the two paths to the food were almost parallel.

A *third* larva was placed at a distance of 11 cm. on the windward side of the food. After a great deal of traveling, making many circles and stopping frequently like the other two, it reached

TABLE VI  
*Showing result of the experiment above indicated*

No. of Larvæ	Time Started June 30, a. m., 1905	Time Arrived	Time Required in Minutes	REMARKS
—	9.05	vials placed	—	
1	9.20	9.30	01	
2	9.27	9.31	4	
3	9.28	9.31	3	
4	9.31	9.37	6	
5	9.32	9.34	2	
6	9.32	11.00-12.00	90+	
7	9.32	9.39	7	
8	no record	9.39	betw. 6-7	
9	no record	9.40	betw. 6-8	
10	no record	9.42	betw. 6-10	
11	no record	10.00	betw. 6-28	
12	no record	9.52	betw. 6-20	
13	9.45	9.51	6	
14	9.45	9.51-10.15	betw. 6-30	
15	9.46	9.51-10.15	betw. 7-30	
16	9.47	9.51-10.15	betw. 8-30	
17	9.47	9.51-10.15	betw. 8-30	
18	no record	9.51-10.15		
19	no record	9.51-10.15		
20	no record	9.51-10.15		
21	no record	9.51-10.15		22 larvæ arrived by 10.15
22	no record	10.15-10.30		
23	no record	10.15-10.30		
24	no record	10.15-10.30		
25	no record	10.15-10.30		
26	no record	10.15-10.30		27 larvæ arrived by 10.30
27	no record	10.30-11.00		
28	no record	10.30-11.00		
29	no record	10.30-11.00		
30	10.40	10.30-11.00	20—	30 larvæ arrived by 11.00
31	no record	11.00-12.00		Observations interrupted
32	no record	11.00-12.00		by lecture at 11.00
33	no record	10.00-12.00		
34	no record	11.00-12.00		
35	no record	11.00-12.00		
36	no record	11.00-12.00		36 larvæ (all) arrived by 12M

Note—When the larvæ started toward the food, they hastened on, stopping once in a while to sway the head about in the air for the purpose of orientation. If the flesh was not reached by means of a direct route, as for instance along the upper side of the vial, the larvæ crawled to the end, then down and

a point farther away from the flesh but on a line with it, and the starting point of the first larva. This process required an hour and four minutes and the larva died at this place apparently from exhaustion.

A *fourth* larva placed  $3\frac{1}{2}$  cm. to one side of the starting point of the first also failed to find the food. Its course led it farther away and finally off the paper.

*Discussion*—From the above two experiments it will be seen that there are two factors involved in finding the food. *First*, the primary stimulation of the larvæ by means of the food. Whatever the nature of this stimulation may be, and whatsoever the internal mechanism involved, the process which underlies the turning of the larva in an effort to draw nearer to the food, may be termed *chemotaxis*. The *second* factor is the swaying of the head from side to side or in an arc of a circle. This the larva does for the purpose of orientation, and the process may be termed, according to Holmes ('05), "Selection of Random Movements," or, according to Jennings ('04), "Trial and Error Movements." Both processes cover the case equally well. The writer has been unable to detect any dissimilarity between the two theories, as applied to the behavior of fly larvæ.

The larvæ stop frequently in their course, sway the head as above indicated, also circle frequently while crawling. The same course may be pursued again or there may be a change in direction which is generally the case after a pause. It is clear that an overproduction of random movements is involved; that a selection is made from these, depending on the force of the stimulation, and that the larvæ are thus guided on their way. On the other hand, it may be said that the larvæ reach the food successfully because they pause frequently in the course and sway the head about in order to *try* the conditions, then when they change the course, it

back to the food. Comparatively few found it necessary to do this, since the more direct route was naturally along the lower side.

The smallest larvæ seemed to have the most trouble in reaching the food. One very small larva (No. 6) remained within a distance of 2 cm. from the flesh for over an hour and a half. No larva left the flesh to return to the smaller vial, though once in a while one started away, but always to return in a few seconds. The larvæ were under observation all day and all evening.

is evident that an *error* has taken place. Therefore, it does equally well to apply the "Trial and Error Theory."

The following quotation from Jennings ('05), p. 475, apparently, makes little distinction, if any, between the two theories just mentioned: "We perform movements which subject us to various conditions, till one is found that relieves the difficulty. We call the process searching, testing, trial, and the like. In the lowest and highest organisms the injurious condition acts as a stimulus to produce many movements, subjecting the organisms to various conditions, one of which is selected."

The use of the terms "*selected*" and "*selection*" which frequently recur in the paper above quoted should not be overlooked without a thought as to their significance. The first impression is that these terms imply *intelligent choice* on the part of the organism, but the author (Jennings) undoubtedly expects the broader interpretation, such as expressed by the term selection when applied to a magnet. This also holds equally well for the theory of the "*Selection* of random movements." There is in reality no intelligent choice involved; the organism responds reflexly to the stimulus, either positively or negatively.

Each of the three theories of animal behavior evidently explains much, but the writer believes, at least in reference to his own experiments cited above and others cited below, that the tropism theory is not sufficient without either the second or third theory, and vice versa. It is still largely a matter of theory whether animal behavior can be so readily explained. Even in fly larvæ we have to deal with what seems to be a death feint, and that in itself leaves much to be explained.

#### *Phototaxis*

Observations were made on the same larvæ used in the first experiment. In the evening of the same day (June 30, 1905,) on lighting the lamp, the larvæ were noticed to leave the flesh at once and hasten toward the side of the vial nearest the light. This took them 4.5 cm. away from the food. Changing the angle between the vial and the light or rolling the tube over always resulted in a readjustment on the part of the larvæ. Moving the



lamp from one side of the tube to the other resulted likewise. Gradually increasing the distance between the vial and the light resulted in a return to the food when a maximum of thirteen feet was reached. On decreasing the distance again, the larvæ once more left the food when a distance of nine feet was reached. This experiment was repeated several times with like results.

The lamp used was an ordinary oil lamp with small (No. 1) wick turned up fairly well. The adjustment to what was apparently the exact point of greatest photic stimulation was very remarkable, as was the almost frantic effort to gain this point when the angle was changed. Here we have an example of positive phototaxis overcoming the action of positive chemotaxis which is surely not a useful reaction.

#### *Stereotaxis*

The larvæ when placed in a receptacle which was ridged, preferred to crawl in the grooves. In one instance larvæ were kept in a bottle which had a convex bottom; on examination later, all were found in a circle wedged in close together around the margin of the bottom, with heads down and posteriors extended. On several occasions larvæ were found crawling in the crevices of the floor, and some of these were wedged in so tightly that it was a task to extricate them without injury to the larvæ.

Positive stereotaxis is a prevailing phenomenon in the lower orders and fly larvæ are no exception.

#### *Geotaxis*

Fly larvæ are positively geotactic, the burrowing habit (?) being very marked. On the other hand, imagines when first emerging from the pupa cases crawl out of the sand and up nearby grasses, remaining there until the wings are spread and dry. The cut showing this also illustrates how the flies cling to the grasses with head downward.

Note—The experiments and observations relating to this paper were conducted at the Ohio State University Lake Laboratory at Sandusky, Ohio, chiefly during nine weeks of the summer, 1905, and a portion of the summers, 1903 and 1904.

The writer is indebted to Prof. Herbert Osborn, Director of the Lake Laboratory and Associates, Profs. F. L. Landacre and J. S. Hine; also to Dr. W. E. Kellicott, Barnard College, for the kind assistance rendered and suggestions offered during the course of these studies.

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# REJUVENESCENCE AS THE RESULT OF CONJUGATION

BY

SARA WHITE CULL

Thirty years ago Bütschli proposed the view, since confirmed by Maupas and others, that the life histories of infusoria run in cycles, and that a period characterized by binary fission is followed by another in which conjugation takes place; this latter process resulting in a thorough reorganization of the exconjugants and a *Verjüngung* or rejuvenescence, which shows itself in a higher rate of cell division and, generally speaking, in renewed life activities. If conjugation does not take place nor an equivalent stimulus be given the organisms they will eventually die of what has been termed "protoplasmic old age."

Hitherto it has been supposed that both cells in conjugation were benefited by the process, a mutual fertilization taking place; but in a series of experiments made by Calkins on *Paramecium caudatum*, the fact was noted that when both exconjugants live, in some cases one is far more vigorous than the other, as demonstrated by the greater number of offspring in one case than in the other.<sup>1</sup> Dr. Calkins suggested that I should examine this point and carry out some other observations that he had already made. The work was done in the zoölogical laboratory of Columbia University in the fall and winter of 1905-06.

Bütschli has pointed out the striking analogy which exists between conjugation and fertilization as it is seen among higher organisms and among those protozoa which show sexual dimorphism. In many of these forms such as the peritrichous ciliates or the coccidiida, there is a marked sex-differentiation in the size and activity of the gametes. Here in fertilization, a more or less passive individual of normal or more than normal size, a macro-

<sup>1</sup> Studies on the Life History of Protozoa. I Arch. f. Entwk., Bd. xv, 1 02.

gamete, completely fuses with a smaller cell of greater activity, a microgamete. The complete union of two cells along with differences in size and activity are characters which distinguish the process of fertilization as usually understood, from the process of conjugation, as seen in forms like *Paramecium*. Both processes agree in having the same essential feature, the union of the nuclei of the two cells. In the different classes of protozoa all steps may be found from conjugation in a general sense to a process exactly similar to fertilization used in a strict sense. Even the maturation phenomena which play so important a rôle in the history of metazoan germ cells are represented in some sort by processes which have been observed in a few protozoa.

In isogamous union, such as that which takes place in *Paramecium caudatum*, two individuals of the same size and approximately equal activities unite for a short time, and the ectoplasm around the mouths of the two organisms fuses to form a sort of bridge over which the nuclei pass. During the maturation phases, previous to this nuclear exchange, the micronucleus of each organism gives rise by division to four or more pronuclei. Two of these are destined to be functional and the others, *corpuscles de rebut*, as Maupas calls them, disintegrate. One of the two functional pronuclei passes into the other organism where it fuses with the stationary pronucleus of that cell, forming one single reorganization nucleus. From this, by repeated division arise the micronucleus and macronucleus of the rejuvenated protozoan. These organisms then proceed to reproduce by ordinary fission.

The species used for the experiments described here was *Paramecium caudatum* and the material was what is known as "wild." Each conjugating pair was taken up in a fine pipette and put into a hollow slide containing some drops of the culture liquid—hay infusion—free from all other protozoa. These slides were then put into moist chambers. In all cases an examination was made after the isolation of the conjugating pairs to see that they had not been separated in the process of handling, for if this precaution were not taken, one could not be sure of dealing with the results of conjugation. On the day following isolation, when, in most cases, the exconjugants were swimming freely through the water, each one

was put into a small glass vial containing liquid similar to that from which they had been taken, and these vials were marked in such a way as to indicate the connection between the various individuals. These vials were examined and the animals counted every few days for a month, and a fresh but not a new food medium was given them each time, the same being used for all the organisms.

Ninety-three pairs of these wild conjugants were isolated at different times and of that number at the end of one month representatives of sixty-five pairs, or seventy per cent, were alive. At least one of the original conjugants remained of each pair, in the majority of cases both had given rise to offspring.

Forty pairs of conjugants from long-continued cultures living in the laboratory on hay infusion were isolated by Calkins (*loc. cit.*) and examined from time to time. Only six pairs, or twelve per cent of these paramecia were represented by living forms at the end of a month. A comparison of these observations with those now made on the "wild" material would seem to indicate that the fertility of conjugation is dependent upon the condition of vitality in the individuals pairing, for, in both cases, the medium was the same. On the other hand, the explanation may lie in the fact that both conjugants had lived in the medium for many months, so that their chemical composition was too similar to produce a new compound by fusion of their nuclei, this new compound being, perhaps, the source of energy for reorganization.

A study of the mortality of these paramecia showed that at the end of one week the strains of both conjugants had died out entirely in six per cent of the original ninety-three pairs. After three weeks had passed thirteen pairs, or approximately thirteen per cent, had died. These facts confirm Calkins' observation that conjugation is by no means always successful in producing rejuvenescence.

The point which interested me chiefly in these experiments was that of double or reciprocal fertility—do both conjugants possess new power and ability to carry on the activities of life, or is but one of them fertilized as is the case among higher organisms? The statistics which were gathered with this in mind show that, at the

end of the month, of the sixty-five pairs then represented by living cells, in twenty-seven pairs, or forty-one per cent, one of the exconjugants only or the offspring from it were alive; in fifteen pairs, or twenty-three per cent, the progeny of one exconjugant was three times as large as that of the other; in six pairs, the descendants of the one were twice as numerous as those of the other organism; and in only five cases had both conjugants given rise to the same number of offspring. The twelve remaining pairs showed a wide disparity in the number of paramecia produced by any two conjugants. The following table shows these results in summarized form:

	PROGENY OF BOTH CONJUGANTS DEAD		PROGENY OF ONE CONJUGANT DEAD, OF THE OTHER ALIVE		PROGENY OF ONE CONJUGANT TWICE THAT OF THE OTHER*		PROGENY OF ONE CONJUGANT SHOWS GREATER VIGOR	
	Number of Pairs	Per- centage	Number of Pairs	Per- centage	Number of Pairs	Per- centage	Number of Pairs	Per- centage
After 7 days	6	6	20	22	29	33	—	—
After 20 days	13	13	25	31	28	35	—	—
After 30 days	28	30	27	41	21	32	48	74

\* Exclusive of cases where the progeny of one exconjugant had died.

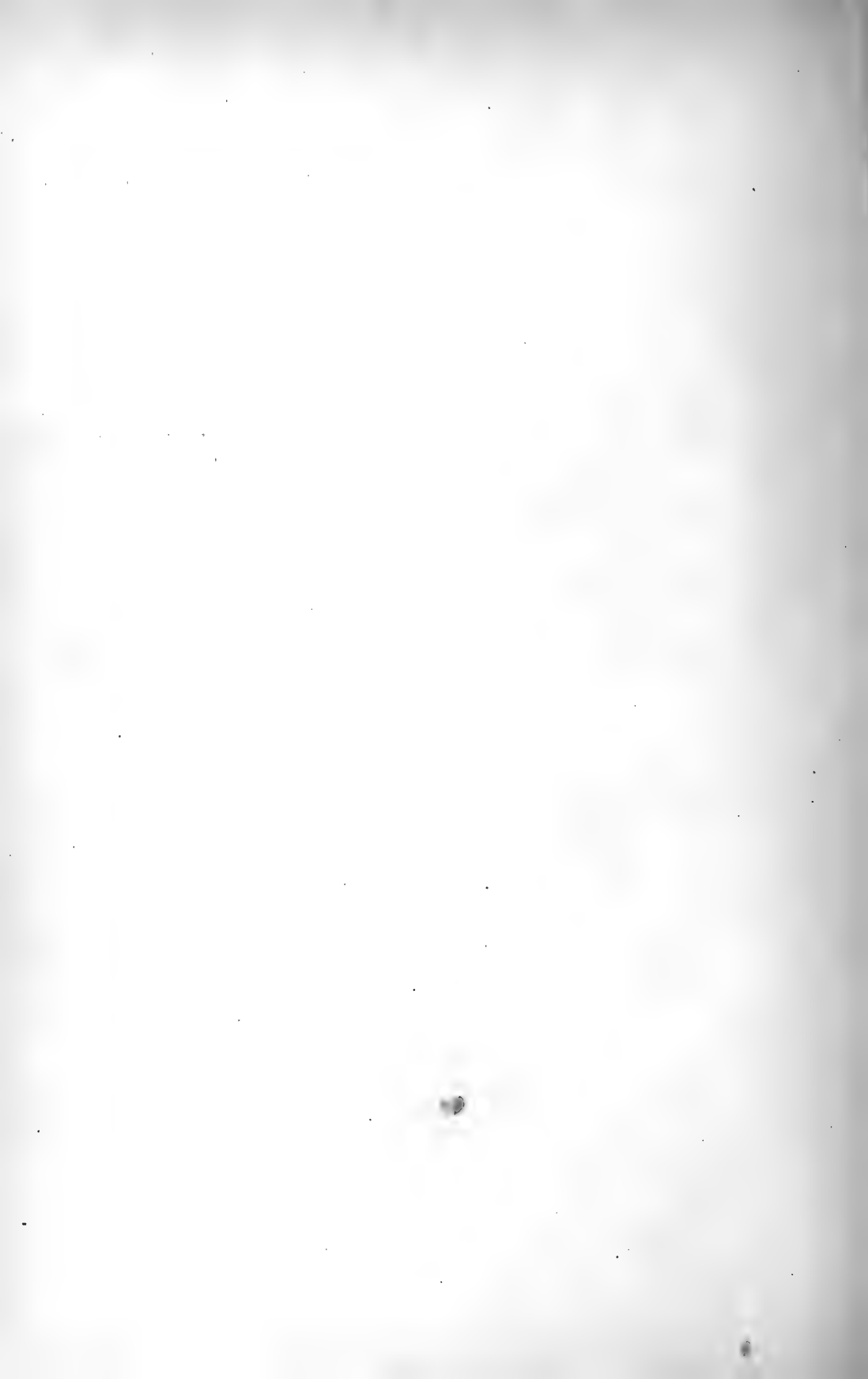
It may be broadly stated that of the sixty-five pairs which I have observed one conjugant either died or left a weak strain in which the descendants were half as numerous and much less vigorous than those of the stronger exconjugant. This striking difference in the restored vitality of the conjugants and their descendants gives strong grounds for the belief that conjugation as seen among these infusoria is really incipient fertilization as seen among the higher forms of life. Here we have indications that one gamete gives up its vitality to and loses its individuality in the other just as the spermatozoön loses its identity in the egg where its presence forms a stimulus to development analogous to the *rajeunissement* and greater activity in cell division which follows conjugation. There is little reason to doubt that a physiological and perhaps a

physical difference exists between the two unicellular organisms which unite in conjugation and a difference of the same nature as that expressed morphologically in the case of *Adelea ovata*, where the male gamete does not fuse with the female but dies after delivering one of its four pronuclei.

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# ARTIFICIAL PARTHENOGENESIS IN THALASSEMA MELLITA

BY  
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WITH SIX PLATES

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## I INTRODUCTION

In two brief abstracts ('05, '06) I have published a few of the results which have been obtained from a study of artificial parthenogenesis in the echiuroid, *Thalassema, mellita* (Conn). I have

there shown that the eggs of this worm can be induced to develop into actively swimming trochophores, in the absence of sperm, by exposure for a few minutes to dilute solutions of acids, both inorganic and organic. Nitric, hydrochloric, sulphuric, carbonic, acetic and oxalic acids were used successfully, and in favorable experiments from 50 to 60 per cent of the eggs thus treated developed into swimming larvæ that could scarcely be distinguished from normal trochophores of a corresponding stage. Continued and more detailed examination of the material has yielded many points of interest which are described at greater length in the present paper.

The experimental part of the work and the observations on the living material were made at the laboratory of the U. S. Bureau of Fisheries, at Beaufort, N. C., during the summer of 1904, while the cytological study was completed in the following summer at the Marine Biological Laboratory at Woods Hole, Mass.<sup>1</sup>

The development and life history of *Thalassema mellita* were first described by Conn ('84, '86), but as most of his observations were made upon the living material alone, his account is superficial and inadequate, and, as has been recently shown by Torrey, many of his descriptions are radically wrong. A careful study, however, of the maturation and fertilization of this worm has been made by Griffin ('96, '99), while the early embryology has been very accurately described by Torrey ('02, '03). With the exception of a brief communication by Kowalevsky ('72), and a note by Cowles ('03) on the rearing of *Thalassema* trochophores into the young worms, there exists no further literature on the development of this genus.

Hitherto, the egg of *Thalassema* has been known to develop only after fertilization by sperm, but my work has shown that it may readily be induced to develop parthenogenetically. It is, moreover, a particularly favorable object for experimental work of this kind.

<sup>1</sup>I wish to express my thanks to the Hon. George M. Bowers, U. S. Commissioner of Fisheries, for the privilege of occupying a table in the Beaufort Laboratory, and to Dr. Caswell Grave, Director of the Laboratory, for many courtesies extended to me. My thanks are also due the Carnegie Institution for the grant of a table in the Marine Biological Laboratory at Woods Hole in 1905.

My purpose in undertaking this research has not been primarily to analyze the physiological processes involved, but rather to study the morphological phenomena concerned in artificial parthenogenesis, and especially, by a careful cytological examination of the material, to compare, as far as possible, the development artificially produced with the normal events leading up to the formation of the larva. My attention, therefore, has not been mainly directed to an investigation of the nature of the action which parthenogenetic agents exert upon the egg, nor to an exhaustive study of the conditions under which such agents act, although in the course of the experimental part of the work a number of interesting facts have been brought out and noted. After having discovered that acids in dilute solutions could cause the formation of swimming larvæ, I did not attempt to extend the method, as it seemed adequate for my purpose.

## II ARTIFICIAL PARTHENOGENESIS IN ANNELIDS

Artificial parthenogenesis has been observed in the case of several other annelids by a number of experimenters. Loeb ('01) first succeeded in causing development of the unfertilized eggs of *Chætopterus* by increasing the osmotic pressure of the sea-water, by the action of KCl and other potassium salts in the absence of the osmotic effect, and by exposure to dilute solutions of HCl. By all of these means he obtained swimming larvæ which he states presented an appearance exactly like that of normal trochophores arising from fertilized eggs. Inasmuch as the changes leading up to the formation of these swimming structures were totally different from normal, developmental phenomena, and as the trochophore stage was apparently reached without visible signs of cleavage, Loeb concluded that normal cell lineage is an entirely secondary phenomenon. The structure of these swimming larvæ of *Chætopterus* was afterwards carefully examined by Lillie, whose observations will be referred to below. In the same paper, Loeb states that he also produced certain changes in the unfertilized eggs of *Phascolosoma* and *Podarke*, the former dividing into 30 to 60 cells, while in the latter only the first cleavage occurred.

Observations on the annelids were next extended by Fischer ('02, '03) to *Amphitrite* and *Nereis*. Mathews ('01) had previously shown that artificial parthenogenesis could be produced in the starfish by mechanical agitation of the eggs, and this Fischer proved to be also true in the case of *Amphitrite*. He found that the eggs of this worm are extremely susceptible to mechanical shock, and can be brought to the "trochophore stage" by squirting them from a pipette after a residence in sea-water of from one-half to one hour. Fischer, furthermore, found that  $\text{Ca}(\text{NO}_3)_2$  is capable of inducing parthenogenetic development in *Amphitrite*, a result which he attributes to the specific effect of calcium ions, although in *Nereis* he thinks the essential factor is the abstraction of water from the egg caused by the increased osmotic pressure of the sea-water. The morphological phenomena concerned in the development of the unfertilized eggs of *Amphitrite* and *Nereis* are nearly as widely divergent from the normal as in the case of *Chætopterus*. The calcium eggs of the former show a totally different appearance from that of eggs fertilized by sperm. Although rarely cleavage may occur in a more or less normal manner as far as eight or twelve cells, the majority of eggs that divide do not go beyond the two-cell stage. Since a larger percentage of eggs reach the swimming condition than undergo cleavage, Fischer was inclined to believe that the formation of the trochophore could take place in the absence of segmentation, a result in harmony with Loeb's conclusion for *Chætopterus*. In *Nereis*, parthenogenetic development is likewise far from normal in character, although cleavage, for the most part very irregular, seems to be of commoner occurrence than in *Chætopterus* and *Amphitrite*.

The true morphological nature of these supposedly normal looking parthenogenetic larvæ of annelids has been clearly elucidated by Lillie ('02), who has shown that the unfertilized eggs of *Chætopterus*, after exposure to salt solutions, pass, without segmentation, through certain phases of differentiation, resembling some of the normal processes, although the resulting ciliated structures are widely different from trochophores arising from fertilized eggs. Since my results in *Thalassema* are utterly unlike those of Lillie, it is necessary to refer to his observations more fully.

Mead ('95, '98b) had already shown that the germinal vesicle of *Chætopterus* breaks down when the egg comes in contact with sea-water; the first maturation spindle forms, and the chromosomes pass into the equatorial plate, but the mitosis is not completed unless fertilization takes place. He also discovered the important fact that the addition of a small quantity of KCl to the sea-water produces the same effect as the spermatozoön, causing the extrusion of the polar bodies, the formation of the yolk lobe, and other changes in the egg preparatory to the first cleavage ('98a, p. 213). Lillie, however, extended these observations, and found that, after exposure for about one hour to solutions of KCl in definite concentration, the unfertilized eggs of *Chætopterus* may undergo a process of cytoplasmic differentiation unaccompanied by cell division, and in about twenty-four hours after the beginning of the experiment give rise to ciliated structures which in some cases more or less simulate the appearance of trochophores. They usually contain but a single nuclear area, and the cytoplasm is differentiated into a ciliated ectoplasm and a yolk-laden endoplasm which are comparable with the ectoderm and endoderm of the trochophore. Since the KCl solutions cause a disintegration and ultimate disappearance of the cell membrane, the naked cytoplasm is left unprotected, and fusion-phenomena between different eggs are of common occurrence, as Loeb ('01) had previously observed, the agglutination being greatly increased by the addition of a small quantity of  $\text{CaCl}_2$  to the K-containing sea-water. In harmony with the results of Loeb, Lillie also observed that the period preceding differentiation of the cytoplasm is characterized by amœboid movements which may exhibit an astonishing degree of activity. If cleavage takes place at all, as it does in some eggs, it rarely goes very far, and only in a small proportion of such eggs does cell division approximate the normal. Division of the cytoplasm unassociated with nuclear division is of common occurrence, the non-nucleated portions of protoplasm always fusing sooner or later with the general mass. A comparison of my own results with the observations and conclusions of Lillie will be made further on.

Results essentially similar to those of Lillie have been obtained by Treadwell ('02) in *Podarke obscura* after treatment of the

unfertilized eggs with solutions of KCl. In this case differentiated, ciliated structures may also arise without segmentation, and pseudo-cleavages, involving only the cytoplasm, are of frequent occurrence. Ciliated embryos, however, may also be produced as the result of a cleavage process, in which both cytoplasm and nucleus are concerned, but here cell-division is quite abnormal. Fusion of separate eggs was observed in *Podarke*, but it is rarer than in *Chætopteris* and fewer eggs unite into a common mass.

Bullot ('04), experimenting with the eggs of an annelid, *Ophelia*, has obtained results which are not in accord with those just cited, in that he has shown that the parthenogenetic larvæ of this worm, produced by solutions of KCl and NaCl, arise *only* from segmenting eggs. How nearly normal the processes of cleavage and differentiation are in this case cannot be determined from the inadequate figures and description given, although he states that "the divisions go on regularly into four, eight, sixteen, and more cells," and that later a blastula of characteristic shape is formed.

Lastly, Scott ('06) has studied the morphological phenomena of parthenogenesis in the eggs of *Amphitrite* which were subjected to the action of salt solutions, especially solutions of  $\text{Ca}(\text{NO}_3)_2$ , and to mechanical agitation. Usually from 5 to 25 per cent of swimming structures were obtained. He found that certain differentiations may occur with or without cleavage and with or without the formation of polar bodies. Cleavage of the egg may take place, but it is generally abnormal, always so in later stages. A ciliated body is produced, which may show more or less extensive cell divisions but usually exhibits no true segmentation; the mass may, however, contain many nuclei. In no instance was anything remotely approaching a normal larva obtained, although certain cytoplasmic differentiations were present, as the development of an ectoplasmic layer, the growth of cilia, and the appearance of vacuoles and pigment.

It is clear, then, that the previous work on artificial parthenogenesis of annelids, with the methods which have been employed, has shown little in common with the processes of normal development, and that at best a ciliated structure has been produced which exhibits certain specialized regions of the cytoplasm but no nor-

mally differentiated organs. That a far more normal result, however, has been obtained in the parthenogenetic development of *Thalassema* will be pointed out in the following pages.

### III MATERIAL AND METHODS

*Thalassema mellita* inhabits the dead tests of the sand-dollar *Mellita pentapora* (Gmelin) and occurs abundantly on the extensive shoals in the harbor of Beaufort, N. C. The egg is a particularly favorable one for experimental work in artificial parthenogenesis. With a little experience one has no difficulty in distinguishing the sexes by the difference in color of the sexual products which show through the semi-transparent body wall, the spermatozoa appearing a milky-white and the eggs a light golden-yellow. Males and females may, therefore, be separated, and it is not necessary to touch the former during the course of an experiment. The full-grown oöcytes are contained in the segmental tubes which fill a large part of the body cavity, and upon rupture of the tubes great numbers of eggs may be obtained in perfectly clean cultures, entirely free from immature eggs, slime and débris of all kinds.

Every precaution was taken to avoid contamination by spermatozoa, and it may be stated at the outset that *in all experiments the control eggs were absolutely negative and never showed a single case of cleavage or differentiation of any nature whatever.*

The female worms were first separated from the males and kept by themselves in a dish of sea-water over night. Before using, they were thoroughly washed in fresh water, as were the dishes and instruments employed in the experiment and the hands of the operator. The body wall was then slit open, whereupon the tubes, gorged with eggs, burst out through the opening. The tubes were first rinsed in sterilized sea-water, in order to remove the blood adhering to their surface, and then snipped off and dropped into a dish of sea-water which had previously been raised to a temperature of 70° or 80°.

The eggs, when first removed from the tubes, are collapsed and pressed out of shape, as a result of close packing in the confined space, and do not become spherical until after fertilization by the



sperm or treatment with the solutions. The unfertilized control eggs, however, for the most part retain the compressed form when allowed to remain in normal sea-water, and finally die in this condition. The eggs, furthermore, when taken from the tubes, are naked, and the failure of the control eggs to ever form a membrane furnishes an additional check on the experiments, for, as will be shown beyond, every egg subjected to the action of the acid solutions throws off a fertilization membrane in all respects identical with the membrane which appears upon the entrance of the spermatozoön.

For the study of sections, eggs and embryos were killed in Wilson's picro-acetic mixture (2 per cent acetic) which gave excellent results. Osmic acid (1 per cent), followed by prolonged immersion in Müller's fluid, proved very satisfactory for later stages, while weak formalin and a mixture of  $2\frac{1}{2}$  per cent formalin and 50 per cent alcohol were useful for the demonstration of cilia in total mounts of the older embryos and larvæ. Sections were stained in iron hæmatoxylin, with or without a counter stain, while Conklin's Delafield's hæmatoxylin gave the best results for whole preparations of the cleavage stages. Most of the sections were cut  $5\mu$  in thickness, and all of the drawings were made with the camera.

#### IV EXPERIMENTAL RESULTS

At the beginning of the investigation, the attempt was made to induce parthenogenetic development by the use of salts, and  $MgCl_2$ ,  $Ca(NO_3)_2$ ,  $KCl$ , and  $NaCl$  were tried. All, however, gave negative results, except in a few cases an irregular fragmentation of some of the eggs was produced, but it never led to the formation of swimming larvæ. In many of these experiments the osmotic pressure of the sea-water was increased, and it would seem, therefore, that parthenogenetic development of *Thalassema* cannot be produced by subjecting the unfertilized eggs to the action of hypertonic sea-water. It should be stated, however, that the range of salt solutions employed was not exhaustive, since early in the work it was found that acids gave promise of better results,

and experiments with salts were discontinued. It is not impossible that favorable solutions of these and other salts might have been found which would have caused development, had the attempt been made to investigate the action of salts in greater detail.

I was also unable to obtain parthenogenetic development by mechanical agitation or by exposure of the eggs to low temperatures. Unlike *Asterias* and *Amphitrite*, the eggs of *Thalassema* show no changes whatever after either gentle or violent agitation, and the low temperatures which Greeley ('02) found to be capable of producing development of the unfertilized eggs of the starfish were utterly ineffectual in bringing about a similar result in the case of *Thalassema*. On the other hand, parthenogenetic development, which was strikingly normal in a great many experiments, took place after treatment of the eggs for several minutes with dilute solutions of certain acids. Nitric, hydrochloric, sulphuric, carbonic, acetic and oxalic acids were employed, and all yielded about equally successful results.

#### I *Acids as Parthenogenetic Agents*

The method of causing artificial parthenogenesis by the use of mineral acids was first elaborated by Loeb and Neilson ('01), who employed a solution of  $3-5 \text{ cc. } \frac{n}{10}$  inorganic acid + 100 cc. sea-water, with an immersion of 3-20 minutes, and in the case of *Asterias* succeeded by this means in bringing about 20 per cent of the eggs to a gastrula stage. They ascribed the result to the specific action of H-ions. Since these initial experiments, inorganic acids have been used with some success as parthenogenetic agents by Loeb and others on the eggs of echinoderms and worms.

The use of  $\text{CO}_2$  in artificial parthenogenesis is due to Delage ('02, '04) who found the method to be remarkably successful with the eggs of the starfish. The eggs were placed in sea-water charged with  $\text{CO}_2$  by means of a "sparklet," and after an immersion in the charged sea-water for about an hour, practically every egg developed into a swimming larva. The larvæ were kept alive for three and one-half months, and were reared to the beginning of

metamorphosis. The oldest larvæ, which were still in an active and healthy state, were accidentally killed, but not before the early stages of the transformation into the starfish had already made some progress. Delage, moreover, observed that parthenogenesis in *Asterias* is independent of the formation of polar bodies and occurs when one, two or no polar bodies have been extruded, a result in harmony with what I have found to be true in *Thalassema*. He states, however, that the treatment must be given at some time during the process of maturation, as it is not effective either before the breaking down of the germinal vesicle or after the formation of the egg pronucleus. It will be seen that this limitation does not hold for the eggs of *Thalassema* which were in every case subjected to the action of the acid while in the germinal vesicle stage.

In my experiments I not only found that the inorganic acids, which had been used in a few cases by previous workers, were efficient parthenogenetic agents, but that certain organic acids as well, namely, acetic and oxalic, were equally successful, if not superior.

A considerable variation was observed in the behavior of the eggs of *Thalassema* in the solutions employed, and, owing probably to differences in internal conditions, possibly in the degree of ripeness, the same solution and the same duration of immersion did not in all cases produce the same result, either in the character of the development or in the percentage of eggs involved in the process. This variability, moreover, was independent of temperature and could not be controlled. It is true, however, in the case of each acid employed, that an optimum solution and an optimum duration of exposure were found which could be relied upon to yield satisfactory results in the majority of experiments. In favorable experiments, which were the rule and not the exception, from 40 to 60 per cent of the eggs underwent development and gave rise to actively swimming trochophores which closely coincided with normal larvæ in appearance.

The following solutions, with the time of immersion, are those that gave the best results; they were, in consequence, most frequently used for obtaining embryos and larvæ:

	Minutes.
17 cc. $\frac{m}{10}$ HNO <sub>3</sub> + 83 cc. sea-water .....	5
15 cc. $\frac{m}{10}$ HCl + 85 cc. sea-water .....	5
10 cc. $\frac{m}{20}$ H <sub>2</sub> SO <sub>4</sub> + 90 cc. sea-water .....	8
12 cc. $\frac{m}{20}$ Oxalic acid + 88 cc. sea-water .....	8
15 cc. $\frac{m}{10}$ Acetic acid + 85 cc. sea-water .....	5

In the case of CO<sub>2</sub>, the gas was passed from a generator into sea-water for ten minutes and the eggs immersed in the charged water for one hour, after which they were transferred to pure sea-water.<sup>2</sup> The result was very satisfactory and usually about 50 per cent of swimming larvæ were obtained by this method.

Although a wide range of solutions and exposures were tested in the case of each acid, in the table on page 12 are placed a few results which are selected from a great many experiments and which will serve as characteristic illustrations.

After determining by experiment the optimum solution and exposure in the case of each acid, satisfactory results were usually obtained by adhering more or less closely to such conditions as experience had proved to be the best, but an examination of the following table will show that the expectation was not always fulfilled. For example, in Nos. 3 and 4, when the same solution of HNO<sub>3</sub> and the same exposure were employed, one experiment yielded 40 per cent of swimming larvæ, while the other gave only 5 per cent; and again, in Nos. 5 and 6, 60 per cent and 25 per cent were obtained, respectively, from an equal exposure to the same HCl solution. It is difficult to assign causes to this seemingly capricious difference in the relative proportions of developing eggs in experiments carried on under conditions as nearly identical as possible.

In addition to the variability of the results obtained in different experiments, where the same solutions and exposures were used,

<sup>2</sup> A "sparklet" apparatus was not available at the time my experiments were made.

No.	Solutions Employed	Time of Exposure	Percentage of Swimming Trochophores
1	17 cc. $\frac{m}{10}$ HNO <sub>3</sub> + 83 cc. S.W.	5 minutes	55
2	18 cc. $\frac{m}{10}$ HNO <sub>3</sub> + 82 cc. S.W.	3 minutes	17
3	18 cc. $\frac{m}{10}$ HNO <sub>3</sub> + 82 cc. S.W.	4 minutes	40
4	18 cc. $\frac{m}{10}$ HNO <sub>3</sub> + 82 cc. S.W.	4 minutes	5
5	15 cc. $\frac{m}{10}$ HCl + 85 cc. S.W.	5 minutes	60
6	15 cc. $\frac{m}{10}$ HCl + 85 cc. S.W.	5 minutes	25
7	16 cc. $\frac{m}{10}$ HCl + 84 cc. S.W.	5 minutes	14
8	18 cc. $\frac{m}{10}$ HCl + 82 cc. S.W.	4 minutes	4
9	10 cc. $\frac{m}{20}$ H <sub>2</sub> SO <sub>4</sub> + 90 cc. S.W.	8 minutes	35
10	12 cc. $\frac{m}{20}$ H <sub>2</sub> SO <sub>4</sub> + 88 cc. S.W.	8 minutes	10
11	15 cc. $\frac{m}{20}$ H <sub>2</sub> SO <sub>4</sub> + 85 cc. S.W.	5 minutes	6
12	12 cc. $\frac{m}{20}$ Oxalic + 88 cc. S.W.	8 minutes	50
13	15 cc. $\frac{m}{20}$ Oxalic + 85 cc. S.W.	6 minutes	45
14	10 cc. $\frac{m}{10}$ Acetic + 90 cc. S.W.	7 minutes	30
15	15 cc. $\frac{m}{10}$ Acetic + 85 cc. S.W.	5 minutes	60
16	15 cc. $\frac{m}{10}$ Acetic + 85 cc. S.W.	6 minutes	60
17	CO <sub>2</sub> passed into water for 10 minutes.	1 hour	50
18	CO <sub>2</sub> passed into water for 20 minutes.	1 hour	00

I was greatly struck with the marked difference in results observed when the strength of the solution or the duration of the immersion was varied by a very slight degree. For example, in a given experiment 60 per cent of the eggs developed into actively swimming trochophores, which could not be distinguished from normal larvæ, after five minutes' exposure to the following solution: 15 cc.  $\frac{m}{10}$  HCl + 85 cc. sea-water. Another lot of eggs from the same female, treated with the same solution, but for 6 minutes instead of 5, yielded only about 5 per cent that underwent any development at all, while in none of the eggs did this proceed beyond the early cleavage stages. Here a difference of but one minute in the time of exposure gave rise to a profound difference in the result, in the one case the solution being adequate to initiate the developmental processes in a majority of the eggs, which then produced apparently normal larvæ, while in the other case only an abortive early development was induced in a very few eggs. Such differences, however, in the relative proportion of larvæ were by no means constant; in Nos. 15 and 16 of the table it is seen that a difference of one minute in the exposure to the same solution of acetic acid had no effect upon the percentage of larvæ obtained.

The following table illustrates a similar variability in cases where the duration of immersion was constant, but the solutions differed very slightly in the degree of concentration:

No.	Solutions Employed	Time of Exposure	Percentage of Swimming Trochophores
1	17 cc. $\frac{m}{10}$ HNO <sub>3</sub> + 83 cc. S. W.	5 minutes	40
2	18 cc. $\frac{m}{10}$ HNO <sub>3</sub> + 82 cc. S. W.	5 minutes	3
3	14 cc. $\frac{m}{10}$ Acetic + 86 cc. S. W.	6 minutes	0
4	15 cc. $\frac{m}{10}$ Acetic + 85 cc. S. W.	6 minutes	55

In each of the two cases cited above, the eggs were taken from the same females and placed in the solutions at the same time, the only difference being that the second solution was stronger than the first by 1 cc. of the dilute acid.

## 2 *Artificial Membrane Formation and Parthenogenetic Development*

The unfertilized eggs of *Thalassema* after transference from the acid solution to normal sea-water, throw off a membrane identical with that which is formed upon entrance of the spermatozoön. The artificial production of a membrane has been observed by former experimenters. O. and R. Hertwig ('87) first discovered that, by the addition of chloroform to the sea-water, the unfertilized eggs of the sea-urchin may be caused to form a fertilization membrane which is entirely normal in appearance.

Herbst ('93) later confirmed the result obtained by the Hertwigs, and found that not only chloroform but several other substances, namely, clove oil, creosote, xylol, toluol and benzol, act in a similar manner, the best results being given by benzol. More recently Herbst ('04) has obtained a normal membrane formation by the use of silver salts. Loeb ('05d, '05e) tested the action of hydrocarbons in this respect and found that the ripe eggs of *Strongylocentrotus* and *Asterina*, when put into 50 cc. of sea-water which has been shaken with 1 cc. of benzol or amylene, immediately form membranes which are identical in appearance with the normal fertilization membrane. By subjecting unfertilized eggs of *Strongylocentrotus purpuratus* to a  $2\frac{1}{2}$  to  $1\frac{1}{2}$  *n* NaCl solution or to a  $2\frac{1}{2}$  *n* cane sugar solution, he also succeeded in causing a membrane formation, but in these experiments the osmotic pressure was so high that the eggs were greatly injured and underwent cytolysis without subsequent development. Solutions of lower osmotic pressure caused development, but not membrane formation ('05a, p. 79).

It should be mentioned that Wilson ('01, p. 533) states for *Toxopneustes* that "some of the magnesium eggs showed a faint ragged membrane, but others were absolutely devoid of a membrane," although he gives no details of his observations on

this point. Hunter ('04, p. 214) also records the presence of a membrane surrounding unfertilized eggs of *Arbacia* after treatment with  $MgCl_2$ .

Loeb ('05), in a series of recent papers, has published the results of experiments which have confirmed my observations on the formation of a membrane after exposure of unfertilized eggs to acid solutions. Although our observations agree as to the power of acids to call forth a membrane formation, certain marked differences occur in our results, and it may be well to compare his experiments and my own in this place. By the use of an improved method, Loeb has succeeded in closely imitating the process of normal development in the unfertilized eggs of *Strongylocentrotus purpuratus*. If the eggs are treated with hypertonic sea-water alone, no membrane is formed, and only a small percentage undergo any development at all. The rate of development of these is much slower than in the case of fertilized eggs and the larvæ arising from them do not rise to the top but swim at the bottom of the dish. By first exposing the unfertilized

eggs, however, to 50 cc. of sea-water to which 3 cc.  $\frac{n}{10}$  of a fatty

acid, *e. g.*, formic, acetic, propionic, butyric, valerianic or caproic acid, are added, for from  $\frac{1}{2}$  to  $1\frac{1}{2}$  minutes, they form a characteristic fertilization membrane when put back into normal sea-water. The membrane was not produced as long as the eggs were left in the acidulated water, nor was it formed when they were taken out a little too early or too late. Eggs treated with the acid alone do not develop, but in a few hours begin to disintegrate, and after twenty-four hours practically all are dead. Subsequent treatment, however, with hypertonic sea-water produces a surprising result. If, after the appearance of the membrane, the eggs are placed in 100 cc. of sea-water, to which 15 cc. of a  $2\frac{1}{2} n$  NaCl solution has been added, for from 20 to 50 minutes, 90 to 100 per cent of the eggs develop with the normal rate of segmentation. "A large percentage of the blastulæ originating from this combination of methods looked perfectly normal, and rose to the surface of the sea-water. Their further development into gastrulæ and plutei occurred with the same velocity as that of the control



eggs, which had been fertilized by sperm; and the larvæ showed an equal degree of vitality" (Loeb '06, p. 168). He did not find, however, that all acids were effective in causing the membrane to appear; with the exception of  $\text{CO}_2$ , all the membrane forming acids were monobasic, organic acids. Mineral acids and dibasic or tribasic organic acids, such as oxalic or citric, were not suitable for the purpose. The acids that gave the best results were the lower representatives of the fatty acid groups.

A comparison of the foregoing with my own observations will show some rather striking points of difference. In the first place, the acids which I found to be capable of calling forth the membrane formation are of a more widely different character, while the response to the acid treatment of the egg of *Thalassema* in throwing off the membrane does not seem to be as narrowly restricted as in the case of *Strongylocentrotus*. Although but few acids were available for use when my experiments were made, those which I employed represent a series of considerable range. Unlike Loeb's experience, I found that the mineral acids,  $\text{HNO}_3$ ,  $\text{HCl}$ , and  $\text{H}_2\text{SO}_4$ , were quite successful with the eggs of *Thalassema*, and, as a matter of fact, some of my best results were obtained with  $\text{HCl}$ . Among organic acids, furthermore, the membrane forming power was not limited to monobasic acids, for one dibasic acid, at least, oxalic acid, yielded about as good results as did the monobasic acetic acid. Contrary to the conclusion to which Loeb came in the case of *Strongylocentrotus*, in *Thalassema*, at all events, it would seem that the acid effect is essential to membrane formation.

It should also be pointed out that the limit between the minimal and maximal exposure of the eggs of *Strongylocentrotus* required for membrane formation is narrower than in *Thalassema*. Although the minimal exposures given by Loeb ('05, p. 122) for several different solutions of acetic acid correspond quite closely at the same temperature with my observations, the eggs of *Thalassema* will form membranes after being subjected to stronger solutions and for a much longer time than is true of the sea-urchin. By referring to the table on p. 101, it will be seen that the optimum treatment in my experiments would have been a decided

over-exposure for *Strongylocentrotus*, according to the figures given by Loeb.

The most striking difference, however, between the behavior of *Strongylocentrotus* and that of *Thalassema* is seen in the subsequent events, after the membrane has been produced by the acid treatment. Whereas, in the former the unfertilized eggs undergo no further development unless exposed to hypertonic sea-water, in *Thalassema* the action of the acid, or perhaps the changes which the egg undergoes as a result of the membrane formation, is sufficient to lead the egg on to cleavage and the ultimate formation of a swimming larva. Treatment with hypertonic sea-water is, therefore, unnecessary for the further development of the unfertilized eggs of *Thalassema*.

During a brief stay at Beaufort, N. C., in June, 1906, I had an opportunity of repeating Loeb's experiments while testing the effect of his combination of methods upon the eggs of *Thalassema*. Although the membrane was formed, as it always is in *Thalassema* after treatment with an acid, the subsequent use of hypertonic sea-water not only did not give rise to larvæ, but actually interfered with the development which would otherwise have taken place with the acid alone when used in the proper concentration. When the unfertilized eggs, after an exposure to an acid, were placed in a solution of NaCl, either no developmental changes occurred and the eggs early disintegrated, or at best only a few early cleavages, usually irregular in character, were produced. I at first tried the same solutions and exposures as Loeb had used with *Strongylocentrotus*, and, after finding them to be futile, I varied the different factors between wide limits. The acids were, of course, used in solutions strong enough to call forth the membrane formation, but too weak to cause the eggs to develop, in order to test the ability of NaCl solutions to bring about development after the membrane had been previously produced by acids. In no case, however, did I succeed in discovering a combination that could cause a normal development and the formation of swimming embryos. The following solutions gave the best results obtained: 10 cc.  $\frac{m}{10}$  HCl + 90 cc. sea-water for five minutes, and

15 cc.  $2\frac{1}{2}$  *m* NaCl + 85 cc. sea-water for from 40 to 50 minutes. The percentage of eggs that divided, after treatment with this combination, was low (about 10 per cent), the cleavage was abnormal, and in no case was a ciliated embryo produced. It should be stated that such developmental changes as did occur in experiments like this must be ascribed to the effect of the hypertonic sea-water, since control eggs showed that the same acid solution, when used alone, was too weak to cause any development.

It was clear from a great many experiments which I made that the use of hypertonic sea-water combined with the acid treatment is not only not an improved method of artificial parthenogenesis for *Thalassema*, but, on the contrary, yields immeasurably poorer results than the method employed in my original experiments. I might also add that the combination of methods gave absolutely negative results with the eggs of the sand-dollar *Mellita pentapora*, which I subjected to a similar treatment.

Loeb ('05e) also found that the eggs of a starfish, a species of *Asterina* occurring in the Bay of Monterey, form a membrane after exposure to solutions of a fatty acid, although a higher concentration is necessary for this form than is required for *Strongylocentrotus*. The eggs, however, do not have to be subsequently treated with hypertonic sea-water, as the process of artificial membrane formation is sufficient to cause development in *Asterina*, and in this respect the case is similar to that of *Thalassema*.

In harmony with the observations of Delage ('02c) on *Asterias*, Loeb also found that the eggs of *Asterina*, which mature normally upon being placed in sea-water, could not be made to develop by exposure to an acid solution until after the breaking down of the germinal vesicle. *Thalassema* eggs, on the other hand, differ markedly in this respect from those of the starfish, for, unless they are fertilized by sperm or acted upon by a parthenogenetic agent, the germinal vesicle remains intact, however long they may lie in sea-water.

V OBSERVATIONS ON THE LIVING MATERIAL

1 *The Unsegmented Egg*

In normal fertilization the spermatozoön enters the oöcyte and lies more or less quiescent in the cytoplasm during the changes involved in the process of maturation which are initiated very shortly after the appearance of the sperm inside the egg. Almost immediately the egg throws off the membrane which soon draws away from the surface and becomes completely detached, while at the same time it becomes spherical, probably as a result of the absorption of water. In the parthenogenetic eggs, the same changes take place. After exposure to the acid solutions, the eggs were in all cases transferred at once to sterilized sea-water, when a typical fertilization membrane became apparent in a very short time. The eggs do not round out as quickly, however, as they do after the entrance of the sperm, but usually in about 30 minutes from the time they are placed in normal sea-water they assume the spherical form, although it occasionally happens that this change is considerably delayed and an hour or more may elapse before they recover from the flattened, compressed condition in which they are when taken from the tubes of the female.

After treatment with the acid and transference to sea-water, the membrane formation was of absolutely universal occurrence and was exhibited by every egg, whether sojourn in the acidulated water was of the proper duration to produce subsequent cleavage and development, or not.

The eggs of my experiments exhibited the phenomena which simulate the appearance of "spinning" activities and which have been described by Torrey ('03) in the normally fertilized eggs of *Thalassema*. If the perivitelline space be examined under a high power, shortly after the appearance of the membrane, excessively fine protoplasmic threads may be seen passing from the surface of the egg to the membrane and varying from time to time in thickness and constitution. These delicate strands persist during the cleavage stages, while some appear to be attached to the polar bodies and give the impression of holding them in place, as Torrey has described. The connections with the polar bodies,

however, are soon interrupted, for the latter usually break away from their original position, and either pass into the cleavage space or float freely about in the perivitelline fluid. Torrey explains the presence of the threads by supposing that, when the membrane separates from the egg, the protoplasm adheres to the corrugations on the inner side of the membrane, and, because of its viscid nature, is drawn out into the threads. If the membrane is merely the denser surface layer of the egg which is mechanically lifted up as a result of the expression of a liquid secreted by the egg, as Loeb ('05e, p. 155) is inclined to believe, the formation of the threads can be readily explained. Fig. 1, which is drawn from the living egg, shows these strands of denser superficial cytoplasm in an egg which has already matured and formed the first cleavage amphiaster.

## 2 *Formation of Polar Bodies*

The first visible change in the interior of the living egg, after transference from the acid solution to normal sea-water is the bodily migration of the germinal vesicle from a position near the center to the animal pole of the egg. This change in location, which may be seen by comparing Figs. 2 and 3, drawn from sections, does not always take place, but it has been observed in a great many cases. In the normal egg the migration does not occur, but the germinal vesicle breaks down near the center of the egg and the first polar spindle later rotates into a radial position, with the outer aster close to the surface, in the usual manner. In the parthenogenetic eggs, however, owing to the outward movement of the germinal vesicle, the spindle arises, as a rule, considerably nearer the surface of the egg.

The extrusion of the first polar body takes place in from 45 to 90 minutes after removal from the acidulated water, thus showing a great retardation of the maturation, since in the normal egg the first polar body is formed in about 20 minutes after the entrance of the spermatozoön. It soon moves away from the surface of the egg and the second arises immediately under it and very shortly afterward, although the interval between the appearance of the two is more variable than in normal maturation.

In the living parthenogenetic egg, the polar bodies are absolutely indistinguishable in position, size, form and other characteristics from those formed after fertilization by sperm. Conn ('86) described the almost invariable division of the first polar body, but as his observations were limited nearly entirely to the living egg, he did not follow the details of the mitotic phenomena. Griffin ('99), however, from a careful study of sections, determined that the first polar body divides by a complete and typical mitosis, although in certain minor details there are signs of degeneration. The same mitotic phenomena and division occur almost without exception in the parthenogenetic eggs, and will be described beyond. Fig. 4, showing the divided first polar body, is drawn from a living egg which had been exposed to the action of acetic acid; the first polar body in this case appeared in 45 minutes after removal from the solution, and the second 15 minutes later.

It is of interest to note that the polar bodies respond to the same divisional stimulus supplied by the acid solutions as does the egg cell itself, as both bodies have been frequently seen to undergo several cleavages. This revived activity of the degenerate polar cells, which may be regarded as an abortive parthenogenesis, results in the formation of a miniature, morula-like cluster of minute cells which, however, soon break away in a mass from the surface of the egg and may persist for some time in the space beneath the membrane. Exposure to the acid solutions restores to a certain degree the energy of division in these rudimentary germ cells and an attempt at development follows. As many as sixteen cells have been counted with certainty in the miniature embryos, and, although I have not been able to determine it in all cases, it is certainly true that some at least of these subsequent cleavages of the polar cells take place mitotically. In Fig. 1 is seen an instance in which at least ten cells have been formed, and Fig. 62 is drawn from a section which has passed through five cells of a cluster; two of these show indications of mitotic activity.

This parthenogenetic development of the polar bodies in *Thalassema* should be compared with the observations of Francotte ('97) on a turbellarian, *Prosthecercæus*; here the first polar

body, which is often abnormally large, is occasionally fertilized by a spermatozoön and develops into a small gastrula.

Although in the great majority of experiments normal polar bodies were extruded by the unfertilized eggs, this was not always true, for in a number of cases the eggs divided and eventually gave rise to trochophores without any external indication of a previous maturation, or after the formation of but a single polar body. It occasionally happened that eggs in the same dish would show all of these conditions, *i. e.*, some would mature normally, some would extrude only one polar body, while others would form none; yet all of these classes of eggs might undergo development and produce larvæ indistinguishable from each other. It was more often the case, however, that in any one dish, in which the eggs had been exposed to the same solution, all the developing eggs would either mature normally, or else throw off only one polar body, or again none at all. As the egg of *Thalassema* is quite opaque, the internal phenomena involved in these changes can only be examined in sections, and their description must, therefore, be reserved for the portion of the paper dealing with observations on the preserved material.

### 3 Cleavage

After the formation of the polar bodies, there are no further signs of change visible in the living egg for some time. The first cleavage does not take place at a definite period after the eggs have been subjected to the action of the solutions, but it may appear at any time, varying from 2 to  $3\frac{1}{2}$  hours, the shorter interval, however, being the more frequent one. The appearance of the first cleavage is not correlated with the time of extrusion of the polar bodies, and a delayed maturation does not necessarily mean a corresponding postponement of segmentation. As the first cleavage occurs after normal fertilization in about 50 or 60 minutes from the time the spermatozoön enters, it is seen that the activities which lead to segmentation are called forth much more slowly in the parthenogenetic eggs.

The early cleavages are closely similar to the normal in a great many cases and in favorable experiments where the optimum

conditions were present, the segmenting eggs could not be distinguished from controls fertilized with sperm, except for the lack of uniformity in the rate of division exhibited by the former, especially during later stages. The rhythm of division is generally more or less disturbed in eggs developing parthenogenetically, and the intervals between successive cleavages are, therefore, less constant than in eggs normally fertilized. All gradations, however, are encountered from cases in which the rate of segmentation closely approximates the normal to those exhibiting nearly every stage of cleavage in the same dish at a given time.

As in the case of the normal egg, the first furrow begins at the upper pole and cuts in somewhat more rapidly here than at the lower, resulting in the formation of two equal blastomeres (Fig. 5). The second cleavage is also equal and gives rise to four blastomeres of exactly the same size. By comparing Fig. 6 with Torrey's Fig. 1B ('03, p. 173), it will be seen that the same relations exist here in regard to the polar furrows as are present in the normal egg. The two upper blastomeres do not quite touch, and one of the polar bodies has passed into the space thus left between them. The four cells, constituting the first quartet of micromeres, are formed at the time and nearly equal the macromeres in size (Fig. 7). The cleavage space from now on increases rapidly in size and frequently one or two polar bodies may be seen lying in it (Fig. 40, *pb*). The origin of the second quartet of micromeres by division of the macromeres, and the unequal division of the first quartet to form the primary trochoblasts, whereby the 16-cell stage is established, as Torrey has described, may be clearly followed in the parthenogenetic eggs and found to be perfectly normal in a great many cases. But beyond this stage I was unable to observe the cleavages with any degree of certainty in the living egg on account of its opacity and the flattening down of the blastomeres during the resting period, a difficulty which Torrey also encountered. A 16-cell stage, drawn from the living egg, is seen in Fig. 8.



4 *Formation of Later Embryo and Larva*

Later cleavage stages, the formation of the blastula and gastrula, and the differentiation of the early trochophore will be considered in the chapter dealing with the observations on preserved material, as most of the developmental changes, which can be determined with accuracy in the parthenogenesis of *Thalassema*, must be made out from an examination of sections and total preparations.

As Torrey has stated (*op. cit.*, p. 187), cilia first appear on the normal blastula with great regularity at four and one-half hours after fertilization, and simultaneously on the prototroch and rosette. Although I have occasionally observed the appearance of the cilia of the prototroch and the apical flagella on the parthenogenetic embryos at the same time, *i. e.*, four and one-half hours after removal from the solutions, it is more usually the case that their formation is considerably delayed and they are not seen for from six to nine hours. In the few experiments where the cilia were first observed at the normal time, the rate of development from the first cleavage onward coincided very closely with the normal rate. In favorable experiments, the cilia show perfectly typical relations; those of the prototroch are at first short and delicate and form a broad band completely encircling the embryo, while the apical cilia soon become quite long and project in front as a pencil of rather stiff straight flagella. Torrey's description of the normal cilia at this stage corresponds in all respects with the observations which I have repeatedly made on the parthenogenetic embryos, and may be quoted: "At first the cilia on the prototroch are uniform in size, but during the differentiation of the trochophore, there appear two bands of longer cilia—one at the upper edge which beat actively, and a narrower one on the lower edge which hang down and move more slowly and indefinitely. Between these two rows the shorter cilia are retained. The long flagella, borne entirely by the rosette, are about 20 in number and when the embryo is actively swimming are carried stretched out in front and bunched closely together, quite as in a pilidium larva. When the animal is at rest the flagella wave about slowly. In the trocho-

phore of about twenty-two hours they are replaced by very short inactive cilia" (p. 190). Compare my Figs. 9 and 10.

All degrees of abnormality, however, may be seen in the ciliation of the parthenogenetic embryos and larvæ. In some cases the apical flagella are entirely wanting or reduced in number, while the cilia on the prototroch may as often fail to form a complete band and appear in irregular clumps or patches, sometimes occurring only on one side; or they may lose every trace of a band-like arrangement and cover more or less uniformly the whole pre-trochal region of the animal (Figs. 11 and 12). In regard to their activity, they may beat in the same way and with about the same vigor as they do in the normal embryo and larva, producing the characteristic spiral movement of the latter. Associated with abnormalities in form and distribution, their movements may also depart widely from those that are typical and lack all apparent coördination. The enfeebling of the stroke may be so pronounced as to render the cilia incapable of causing any bodily movement of the embryo. I gained the impression that functional derangement of the cilia was always correlated with morphological abnormalities of the embryo, and that the farther the embryo departed from the normal in structure, the more erratic were the movements of the cilia.

As Torrey has described, the embryo is at first entirely separated from the egg membrane, but later through the elevation of the surface in these regions, the rosette and primary prototroch become pressed against the membrane which is then punctured by the cilia (p. 187). In the parthenogenetic embryos, especially when the relations are more nearly normal, the same perforation of the membrane by the cilia takes place. This, however, is not always the case. Sometimes the perivitelline space is so greatly enlarged and the membrane in consequence so far removed from the surface of the embryo, that the cilia only touch it at their outer ends, if they reach it at all; or, again, although the membrane may lie close to the embryo, the cilia fail to puncture it and are bent down, being held in this condition until they are released by the rupturing of the membrane (Fig. 13).

As already stated, the movements of the cilia are usually quite

normal in character and their stroke apparently as vigorous as in embryos and larvæ produced from fertilized eggs, yet I have never observed a single instance where the trochophores rose to the surface of the water. They invariably swim close to the bottom, if not in actual contact with the dish. This peculiarity in behavior has been observed with remarkable constancy by most experimenters on artificial parthenogenesis, although Delage in the starfish, after treatment with  $\text{CO}_2$ , and Loeb in the sea-urchin, by the use of his combination of methods, has succeeded in obtaining larvæ that rise in the usual manner to the surface. In *Thalassema*, however, none of the methods which I have employed has produced trochophores that are free from the abnormality in question.

Unless the parthenogenetic larvæ were isolated, they would rarely live over 36 hours, for the eggs which had failed to develop were soon attacked by bacteria, much sooner in fact than the control eggs which had not been exposed to the solutions, and contaminated the dishes to such a degree that the larvæ speedily succumbed. It was, moreover, extremely difficult to separate the larvæ, as they did not rise to the surface, and had to be picked out individually from among the non-developing eggs with a very fine pipette. The trochophores, however, that were isolated were kept in separate dishes and the water frequently changed, yet in no case did I succeed in rearing them longer than a little over three days. The oldest trochophore raised lived about 80 hours. There was no appreciable advance in development, however, beyond the second day, as the larvæ seemed to enter upon a stationary period at that time. As they grew older, their movements became more and more feeble and irregular, and they gradually disintegrated, the body finally rupturing and the protoplasm flowing out.

### 5 *Abnormalities of Cleavage and Behavior*

Although in experiments where the optimum conditions were present the great majority of the dividing eggs segmented in a normal manner, an endless variety of irregular cleavages were encountered, especially in cases where the strength of the solution

employed or the duration of immersion was not such as to yield the best results. Many of these abnormalities are similar to those which have been described by others (*cf.*, especially Wilson, '01) and need not be spoken of in detail here. That such abnormal cleavages lead to the formation of ciliated structures is clearly indicated by the fact that the percentage of all eggs dividing, both normally and abnormally, nearly agrees with the percentage of swimming embryos which are later found in the same culture. Many such counts were made, and the correspondence in percentage was found to be remarkably close. I have, moreover, frequently isolated abnormally segmenting eggs and directly observed them to develop into ciliated, cellular structures. It is extremely doubtful, however, whether eggs that divide irregularly ever undergo a later regulation and produce normal embryos, for all the ciliated structures which were raised from isolated, abnormally segmenting eggs departed more or less widely from normal forms.

Previous experimenters on artificial parthenogenesis of annelids have observed that ciliated structures may arise from unsegmented eggs by a process of progressive cytoplasmic differentiation which takes place in the entire absence of cleavage [Loeb ('01) and Lillie ('02) in *Chætopterus*, Fischer ('02, '03) in *Amphitrite* and *Nereis*, Treadwell ('02) in *Podarke*, and Scott ('06) in *Amphitrite*]. In *Thalassema*, on the other hand, it is undoubtedly true that eggs which do not divide never undergo further differentiation and never form ciliated structures (*cf.* Bullot, '04). Without exception every swimming embryo observed possessed a well-marked cellular structure, and eggs which had failed to segment after residence in acidulated water, when afterwards isolated, in no instance gave rise to a differentiated body.

Certain common types of abnormal cleavage were constantly met with, *e. g.*, every degree of inequality in size of the blastomeres formed by the first and second cleavages was found, while eggs were frequently observed to fall at once into three or four cells at the first division. The trefoil stage, in fact, seemed to be characteristic of certain solutions, in which a great preponderance of eggs exhibiting this abnormality was noted. An unequal division of the eggs at the first cleavage was occasionally followed by division

of the larger blastomere in a plane parallel to the first, so that three nearly equal cells were formed in a row, as shown in Fig. 14. One of the commonest abnormalities, and one which might be observed at any stage of the development, was due to the failure of one or more cells to undergo cytoplasmic cleavage when the nucleus divided, and, as a result of this condition, large multinucleated cells might be found in embryos of all ages. Some examples of such cases as these will be referred to below.

Owing to departures from the normal type of cleavage, young blastulæ are occasionally produced in which the cleavage cavity is not closed; this results in the formation of a cylindrical embryo which is open at both ends. An optical section of this modified blastula, showing the cells radially arranged around the cavity, is drawn in Fig. 15.

Although gastrulation usually takes place in parthenogenetic development in quite a normal manner, disturbances in the process do occur and produce a great variety of pathological embryos. Many degrees of incompleteness in gastrulation may be seen, the extreme case being one in which only a very few entoblastic cells sink into the cleavage cavity. Such embryos, although they may be ciliated and externally resemble trochophores, are found upon sectioning to be merely large, hollow, spheroidal bodies in which the enteron is represented by a few scattered entomeres.

Amœboid activities, which have been observed so commonly in the parthenogenetic eggs of echinoderms and worms, are very rare in the eggs of *Thalassema*, and when they occur at all, the formation of pseudopodia is not extensive and takes place very sluggishly indeed; Fig. 16 represents about as pronounced a case as I have seen in my experiments.

Fusion phenomena, which were so conspicuous in the experiments of Loeb ('01) and especially in those of Lillie ('02), after treatment of the unfertilized eggs of *Chætopterus* with KCl and  $\text{CaCl}_2$  solutions, were entirely absent in *Thalassema*. In *Chætopterus*, eggs may fuse into masses which show certain differentiations and form giant, ciliated structures and double, triple, quadruple, etc., monsters. Lillie, for example, has described fusion masses, into the composition of which about 100 eggs had entered, and

these through amœboid activity gave rise to a "veritable wilderness of pseudopodia." Such fusions are rendered possible by the destruction of the egg membrane, after treatment with salt solutions, especially solutions of  $\text{CaCl}_2$ , and, with the heightening of amœboid movements, the naked masses of protoplasm show a strong tendency to adhere whenever they come in contact. The acid solutions, however, which I have used, never cause disappearance of the membrane in *Thalassema* eggs, and this may explain, in part at least, why fusion phenomena are entirely absent. The eggs in which a membrane formation has been called forth never adhere, but during the later stages of disintegration they may become attached by their membranes to form clusters like frog spawn; here, however, there is no protoplasmic fusion.

#### VI OBSERVATIONS ON THE PRESERVED MATERIAL

The internal changes involved in the artificial parthenogenesis of *Thalassema* have been followed as closely as possible, and by means of sections and total preparations many stages have been examined throughout the entire period of development covered by the material.

In the study of the preserved material, however, serious difficulty is encountered in the attempt to determine the proper sequence of changes taking place in the eggs and embryos, since, as has been pointed out, the rate of development varies so greatly in different eggs, even in the same culture, that a large number of stages may be represented in each sample lot preserved. Furthermore, the living egg can only be used as a control to a limited extent, as it is too opaque to allow of any detailed examination of internal changes, and an attempt to reconstruct successive stages of development from a study of preserved material alone is attended with more or less unsatisfactory results. Nevertheless, with respect to most points, this uncertainty is reduced to a minimum, and in cases where the parthenogenetic embryos coincide in structure with those produced from fertilized eggs, it is fairly safe to take for granted that the observed stages of development have followed in their normal sequence. It becomes much greater,

however, when we are dealing with unusual or abnormal conditions for which no control can be had from living eggs, and here conclusions can only possess a greater or less degree of probability according to the circumstances of the particular case.

### I *The Oöcyte and the Maturation Divisions*

The full-grown oöcytes, when removed from the tubes, possess a large nucleus placed somewhat eccentrically. The cytoplasm is heavily charged with yolk spheres, which stain a deep black with hæmatoxylin and are present throughout the entire cell body, except for a peripheral and a perinuclear layer of yolk-free cytoplasm (Fig. 2). The nucleus is filled with a granular reticulum which takes the plasma but not the chromatin stains at this time. The large vacuolated nucleolus persists until the breaking down of the germinal vesicle, when it gradually dissolves and disappears. The reduced number of chromosomes (12) lie scattered in the nucleus, usually near the membrane, and appear as coiled or twisted granular rods which in some cases are clearly seen to be double or to have the form of rings, or loops, or crosses. As a rule, they stain quite black with hæmatoxylin and stand out conspicuously in the lighter, granular reticulum (Fig. 2). During the early prophases of the first maturation mitosis, the chromosomes undergo a concentration, take up the stain more intensely, and assume the variety of tetrad forms which have been minutely described by Griffin ('99). In this condition they approach the developing asters and finally enter the spindle.

The formation of the membrane and the filling out of the egg to the spherical condition, after treatment with acidulated water, have already been described. It has also been mentioned that in many cases the nucleus migrates bodily to the animal pole, after the eggs have been returned to normal sea-water (Fig. 3), although this change in position does not always occur. The development of the amphiaster for the first maturation division can only be faintly observed in the living egg, but the process may be followed in great detail from an examination of sections. The earliest material I have in which the asters are unquestionably present was fixed 15 minutes after the eggs were exposed to an

acid solution. Since they first become evident in normal eggs about 3 minutes after entrance of the sperm, their appearance is much retarded in the parthenogenetic eggs. They are situated very close to the nuclear wall, and each contains at its center a deeply stained centrosome or centriole (Fig. 17). As soon as the two asters can be discovered at all, they are invariably found some distance apart, and, like Griffin, I have been able to find no evidence that they arise by division of a single aster whose products afterwards diverge. In some cases Griffin (*op. cit.*, p. 590) observed a bipartite condition of the centrosomes even at this early stage, but in my acid treated eggs I have not found them divided until a somewhat later stage. I have also been unable to discover in eggs which have been exposed to acid solutions any trace of the "secondary asters" which Griffin (p. 590) has described as being present in both fertilized and unfertilized eggs, but disappearing in the former in about three minutes after entrance of the sperm. The rays of the asters are at first few in number and excessively delicate, but, as they develop, they appear to grow into the nuclear membrane, flattening it and throwing it into folds and wrinkles, until they finally rupture it and enter the nuclear area (Fig. 17). As the formation of the amphiaser for the first polar mitosis in normal maturation has been carefully described and figured by Griffin, and as these changes are usually the same in every detail in unfertilized eggs which have been subjected to the action of acid solutions, an elaborate account is not necessary here.

During the continued invasion of the nucleus by the astral rays, the nuclear membrane gradually dissolves away, and the granular reticulum, now staining a dark blue with hæmatoxylin, is left in the cytoplasm where it afterwards disappears. Some of the rays, which are thicker and stain more deeply than the rest, appear to attach themselves to the chromosomes, the so-called "traction fibers." The centrosomes are now distinctly double, as may be seen in Figs. 18 and 19, which show stages in the formation of the amphiaser. The definitive spindle (Fig. 20) carrying the chromosomes in the equatorial plate, now swings into a radial position in such a way as to bring the outer aster close to the surface of the egg. The outer rays of this aster shorten, while those situated more



laterally curve backward and cross the corresponding rays of the inner aster in the plane of the equator of the spindle, as in the preceding figure. The chromosomes (tetrads), which show during the prophase the varied forms described by Griffin, undergo a concentration, and when seen in the equatorial plate present a more or less uniform type consisting of a cross, with a pair of thick broad arms in the equatorial plane and a pair of narrower perpendicular arms. A split is frequently seen in the latter, and sometimes the transverse arms also appear to be divided in the middle by a faint line. According to Griffin, the split in the transverse arms of the cross corresponds to the original, longitudinal division of the spireme segment, and the first maturation division is, therefore, equational or longitudinal. This may be the case, but after a careful examination of the tetrads in both unfertilized and fertilized eggs, I am unable to find any definite basis for a determination of the character of the first division. The variability of the form of the crosses renders the identification of either the equatorial or the polar arms with the longitudinal axis of the primary rods extremely uncertain. At the first mitosis, the crosses are drawn out into ellipses, which then divide, the dyads passing to the poles either as V's or double rods. The separation of the V's at the apex takes place at the second maturation division, which Griffin interprets as a reducing division. The outer pair of centrosomes and group of dyads become contained in a small projection or knob of clear, yolk-free cytoplasm, which by constriction around its base is eventually cut off as the first polar body (Figs. 21 to 23).

Before the close of the first mitosis, however, the two inner centrosomes diverge in a direction nearly transverse to the axis of the original spindle; a minute spindle appears between the two, and around each centrosome a new system of delicate rays is formed (Fig. 21). The dyads left in the egg lie at first on the outer side of the spindle which now rapidly elongates and rotates through about  $90^\circ$  of arc until it assumes a radial position with the outer aster immediately under the point on the surface where the first polar body was cut off (Fig. 22). The dyads, in the form of double rods, arrange themselves in the equatorial plate with the long axis across the spindle, as seen in the preceding figure, and

during the anaphase the halves of the dyads move to the poles (Fig. 23). A comparison of Figs. 21 to 23 with Griffin's Figs. 18, 21, 25, will indicate their close identity. The second polar body is constricted off in the same manner as the first and contains twelve single chromosomes, while the same number is found in the egg. I have occasionally observed a doubling of the inner centrosome of the second spindle, as described by Griffin; Fig. 23 shows a case in point. The twelve chromosomes left in the egg (Fig. 24) pass at once into vesicles which at first only partially fuse to form the egg nucleus, while every trace of the inner centrosome and its accompanying rays soon disappears (Fig. 25).

As described by Conn ('86) and by Griffin ('99), the first polar body invariably divides, and, as the latter has determined, it does so by a complete mitosis. The same division has been repeatedly observed in the parthenogenetic eggs, as shown in Fig. 4, which is drawn from a living egg, and also in Figs. 21, 23, 25 and 45 where the minute spindles and rudimentary asters with their centrosomes are clearly seen in section.

In the fertilized egg, the second polar body never exhibits an attempt at division (Griffin, p. 615), but observations on living eggs, which have been exposed to acid solutions, show that all three polar bodies continue to divide until a cluster of miniature cells have been formed, as already described (Fig. 1). Although I cannot state that all of these divisions take place mitotically, I have repeatedly observed distinct indications of spindles in sections of the groups of polar cells; Fig. 62 shows, for example, a spindle in two of the five cells present in the section.

Up to this point the history of the parthenogenetic egg is identical with that of the egg fertilized by sperm, except for the absence in the former of the spermatozoön and its aster, and all of the details of the process of maturation which Griffin has so carefully worked out may be easily verified on the eggs of my experiments. From now on, however, a special description of the changes taking place in the parthenogenetic eggs becomes necessary, for the absence of the sperm nucleus and asters radically alters certain subsequent events, and a special account, quite different from that of the usual phenomena, must be given of the origin of the cleavage nucleus and amphiaster.

## 2 *Origin of the Cleavage Nucleus and Amphiaster*

After extrusion of the second polar body, the egg nucleus moves toward the center of the egg and soon loses its irregular contour by the complete fusion of the chromosomal vesicles which entered into its composition. There now follows a long resting period, and during this pause, which may last for from one to two hours, or even longer, the nucleus increases considerably in size (Fig. 26).

The first indication of the preparation for cleavage is seen in the simultaneous appearance of two delicate asters which are situated opposite each other and immediately outside the nuclear membrane (Fig. 27). They lie in a plane approximately perpendicular to the axis of the egg, and in the center of each aster a distinct centrosome is visible. There is not the slightest evidence that the cleavage asters, for such these are destined to be, are derived by division of a single primary one; when they are first seen they lie at a considerable distance from each other, usually on opposite sides of the nucleus. There is, furthermore, no doubling of the centrosomes at this time, and no intermediate stages are found which would indicate a division and separation of the asters. Fig. 27 illustrates the almost invariable condition of the egg nucleus when the asters are first discovered. My observations agree with those of Wilson ('01) on the magnesium eggs of *Toxopneustes* in that they show clearly that the cleavage centrosomes lie outside the nuclear membrane but immediately upon it, although Wilson is strongly inclined to believe, from the evidence presented by his sections, "that the cleavage amphiaster arises by division of the single center" (p. 564). He first observed a single centrosome, surrounded by a conspicuous aster, lying outside the intact nuclear membrane at one pole of the nucleus; all intermediate conditions were found between this stage, through a monaster stage, to the complete establishment of the dicentric figure, and, while recognizing the possibility of being misled by the nature of the material, he felt justified in regarding the amphiaster as the product of division of the primary monaster. As the evidence on which he bases his conclusion for *Toxopneustes* is not presented

by *Thalassema*, I have not been able to arrive at a similar interpretation for the eggs which I have studied. It should also be mentioned in this connection that Hunter ('04) gives an account of the origin of the cleavage asters for the parthenogenetic eggs of *Arbacia* which is in harmony with Wilson's view. In a brief note he merely makes the statement that "a small aster with central dark body (parthenocentrosome) appears in contact with exterior of nuclear membrane. This divides to form the amphiaster" (p. 214).

The question of the origin of the cleavage centrosomes of *Thalassema* will be referred to in a later section of this paper, but I have little doubt that they are new formations. There is not the least indication of a continuity between the egg center and the cleavage centers, the former totally disappearing at the close of maturation, and I am not inclined to accept the view that the egg center persists, without at least some evidence of the fact.

### 3 *The Cleavage Stages*

The completion of the first cleavage takes place entirely in accordance with Griffin's description of the process in the fertilized egg, except that only 12 chromosomes (the reduced number) are derived from the spireme of the nucleus.

After the stage drawn in Fig. 27, in which the nucleus is seen somewhat drawn out toward the asters, the nucleus elongates still further and becomes fusiform. As the membrane disappears at the poles, the inner rays of the asters invade the nuclear area and form the spindle, some of the fibers appearing coarser and wavy and attached to the chromosomes; these are the so-called "traction fibers." With the development of the rays and spindle, a centrosphere surrounding each centrosome appears, and, as it rapidly increases in size, it becomes reticulated (Fig. 28). At the time of metaphase the centrosomes divide and pass toward the outer side of the spheres.

*Twelve rod-like chromosomes are derived from the spireme thread*, which is left in the equatorial plane of the spindle after the disappearance of the nuclear membrane. Their number, which can be readily determined in sections of the equatorial

plate, is shown in Fig. 29. The chromosomes divide typically, pass to the poles, and in the telophase are converted into vesicles from which the daughter nuclei arise by fusion (Fig. 30). The division of the egg into two equal blastomeres now takes place. During the anaphase, the centrosomes at each pole begin to separate, and soon delicate radiations begin to arise around each as a new center before the original rays have disappeared (Fig. 30).

The difficulties in the way of tracing the cell lineage of the parthenogenetic eggs are so great that a detailed comparison with the normal cleavage and a determination of the origin and fate of the constituent cells of the embryo are rendered practically impossible. Owing to irregularity in the rate of division and the possible presence of greater or less abnormalities at all times in development, the arranging of stages that appear to be normal in their proper sequence can only be attended at best by uncertain results. I shall, therefore, not attempt a continuous account of the development as far as I have been able to follow it, as any reconstruction of stages would be quite arbitrary and would rest almost entirely on the identification of individual embryos with normal ones of a corresponding degree of differentiation. Although in favorable experiments, where optimum solutions were used, I have met with no such "carnival of development" as many other experimenters in artificial parthenogenesis have observed, and although the proportion of embryos that are either normal or nearly so is very large, nevertheless abnormalities of all kinds are of not infrequent occurrence, and the determination in a given case as to whether an embryo is normal or not is by no means easy. But in spite of these difficulties, I am convinced that the parthenogenetic eggs in a great many cases undergo a normal development and exhibit the usual processes of differentiation leading up to the formation of the swimming larva. The only differences in such cases between the organisms which are produced parthenogenetically and those arising from fertilized eggs are to be found in the numerical relations of the chromosomes, as I shall point out beyond, in the rate of development and in the fact that the parthenogenetic larvæ do not rise to the surface of the water when they begin to swim.

The establishment of the second spindles in quite a normal manner may be seen in Figs. 31, 32 and 33, and in the latter the centrosomes of one of the cells are clearly double. This bipartite condition of the centrosomes has been observed repeatedly during mitosis at all stages of the development (Figs. 35, 36, 40), but I have not been able to demonstrate their persistence through the resting period from one generation of cells to the next. After the nuclei return to the resting condition, every trace of asters and centrosomes disappears, and their presence is not revealed again until the early prophase of the next mitosis. I am inclined to believe that, although the centrosomes may divide and diasters arise at the close of a mitosis, these totally disappear, and that the asters for the next cleavage are new formations brought into evidence when the activities of the cell which bring about division are renewed.

Fig. 34 is drawn from a four-cell stage during the resting period, and Fig. 35 from an eight-cell stage, showing four of the cells in mitosis; the early formation of the cleavage cavity is seen in the latter figure. Later stages in the formation of the blastula are illustrated in Figs. 36 to 40, in each of which one or more cells are taken in mitosis. Fig. 39 is probably somewhat abnormal, as it shows an unusually large blastocœl, a not infrequent condition.

In Fig. 36, the section has passed through the equatorial plate in the cell indicated at *a*, and here the twelve chromosomes can be easily counted. As will be emphasized beyond, the reduced number of chromosomes has been repeatedly determined in cells of blastulæ and gastrulæ (Fig. 41, *a*) and the evidence is, therefore, perfectly clear that, except under certain abnormal conditions which will be referred to, a restoration of the normal number of chromosomes does not occur.

#### 4 *Gastrulation and the Formation of the Trochophore*

Gastrulation, which according to Torrey ('03) takes place about seven hours after fertilization, is considerably delayed in parthenogenetic development and occurs at from eight to twelve hours after exposure to acid solutions. The process is of the embolic type, and the insinking of the entoblastic plate can be

observed in the living embryo as well as followed from an examination of sections. It seems to be perfectly normal in character in a great many parthenogenetic embryos, although I have not been able to compare the origin of the entoblastic plate with its normal cell lineage. Sections, however, show a close correspondence with Torrey's description. The lower pole of the blastula flattens, while the upper becomes somewhat arched, and the entoblastic cells elongate and sink bodily into the cleavage cavity which soon becomes nearly filled with them. Early stages of gastrulation are illustrated by the sections drawn in Figs. 40 and 41, which closely correspond with Torrey's Fig. 10 (p. 232). After sinking in, the entomeres multiply rapidly and form a rounded mass which withdraws a little from the body wall. The enteron now consists of a thick epithelium surrounding a small lenticular cavity which later becomes greatly enlarged, while the cells composing its walls are densely filled with yolk spheres. Fig. 42 shows the entoblastic mass with its early cavity, and, although the section does not pass through the blastopore, it is very similar to Torrey's Fig. 6A (p. 207).

The blastopore, an elongated slit (Fig. 9), shifts from the lower pole, where it first appears, to the future ventral side, until it comes to lie close under the prototroch. Although I have not followed its history in all particulars, the changes which it undergoes in the parthenogenetic embryos evidently agree with the account given by Torrey, who describes the enlargement of the anterior end of the slit-like opening and the persistence of this portion to form the mouth, while the posterior portion closes by approximation of its sides.

After gastrulation has taken place, an invagination of the ectodermal cells around the blastopore occurs and gives rise to the œsophagus (Fig. 43), the blind end of which abuts against the closed enteron. In the formation of the œsophageal invagination, three large cells, the œsophagoblasts, are distinguished from the rest by their size and come to occupy a definite position in the wall of the œsophagus, as described by Torrey (p. 205). The continuous history of these cells has not been followed in my material, but that the same specialized cells are present in the parthenogenetic

embryos is clear from an examination of Figs. 41 and 43 (cells designated at *oes*) which should be compared with Torrey's Figs. 10D and 6B, respectively.

The enteric cavity becomes secondarily divided into stomach and intestine by a partition, consisting of a double row of cells, which grows across from the dorsal wall and completely divides the stomach from the intestine except on the ventral side where an opening persists. The division of the archenteron into two cavities by this septum is well shown in Fig. 44, although the section is a horizontal one and does not show the œsophagus and the communication between the stomach and the intestine. The ectodermal œsophagus secondarily acquires an opening into the stomach, but the anus, which is not formed until a very late stage in the normal development, has never been observed in the parthenogenetic larvæ.

Fig. 44 is drawn from a CO<sub>2</sub> trochophore, which was killed 31 hours after the eggs had been treated with carbonated water. Although larvæ were raised for a considerably longer time than this, differentiation rarely proceeded beyond the condition indicated in the preceding figure. In the trochophore here represented, in addition to the digestive tract which is differentiated into mouth, œsophagus, stomach and intestine, the prototroch is present, and the high columnar cells bearing the apical flagella are distinctly shown. A few cells of the larval mesenchyme (ectomesoblast), stippled in this and the two preceding figures, are seen scattered between the body wall and gut, to both of which they are adhering.

I have not observed the definite ventral neural ciliated region described by Torrey, but occasionally more or less irregular patches of cilia have been found in this portion of the larva, as they have been, in fact, in other regions as well.

### 5 Rudimentary Cells

The remarkable rudimentary cells whose origin and fate Torrey has so accurately described in *Thalassema*, are also recognized in the parthenogenetic embryos, although I hardly think they are as numerous here as in embryos raised from fertilized eggs. These



cells arise, according to Torrey, early in the cleavage, and, wandering into the blastocœl, are seen lying on the cells of the entoblast into which they soon sink (Figs. 40, 41, *rc*). Here they become quickly absorbed and disappear. As long as they are present they may be easily recognized by their small size and the contracted condition of their chromatin. In Fig. 40 is also seen a small cell *pb*, which is probably a polar body that has passed into the cleavage cavity; its cytoplasm has a faintly radiate appearance suggesting mitotic activity.

### 6 *Abnormal Maturation Phenomena*

In an earlier part of this paper it has been stated that in some instances the unfertilized eggs of *Thalassema*, after an exposure to acid solutions, failed to extrude the second polar body, and in still other cases that neither body was formed. It was frequently observed, however, that such eggs segment and produce embryos and larvæ indistinguishable from those arising from eggs that have thrown off both polar bodies. These abnormalities were not associated with special solutions or external conditions of the experiment, but they appeared at any time, and even with the optimum solutions.

It was of importance to determine the internal phenomena present in eggs showing these abnormalities of maturation, and an examination of sections from material preserved when the unusual conditions were met with has brought to light several interesting facts. In many of these cases, it is undoubtedly true that either the first or the second polar mitosis, or possibly both in some instances, may take place entirely inside the egg and without accompanying cytoplasmic cleavage, and in this submerged condition give rise to resting nuclei.<sup>3</sup>

#### *a* Absence of the Second Polar Body

In the eggs which extrude only the first polar body, the second spindle fails to assume its usual position, and instead of rotating in such a way as to bring one pole to the surface, it sinks down

<sup>3</sup> Cf. the observations of King ('06) on the retention of one or both polar bodies in unfertilized starfish eggs after compression.

until it comes to lie much nearer the center of the egg. In this unusual situation, it completes the mitosis and gives rise, without much doubt, to two resting nuclei. Fig. 45 shows a fortunate section in which the first polar body is present and the second spindle lies deep within the egg; the chromosomes which are single rods are in a late anaphase and have already reached the poles. The spindle, which is cut throughout its entire length, as both centrosomes are present in the section, is perfectly normal in all respects save its position and unusual length. The objection cannot be made that this might be the cleavage spindle, as the two are entirely different in appearance. Although the proper sequence of stages cannot be determined with absolute certainty from a study of the preserved material, there can be little doubt that this particular mitosis, and many others like it which I have seen, is taking place inside the egg. All of the eggs on which I have based this conclusion belong to a single experiment, No. 29 of my notes, in which not an instance of the formation of the second polar body was observed while the eggs were alive; sample lots from this experiment were killed at intervals during the maturation period, and I have sectioned several stages of the series. My attention being attracted to the peculiarities of maturation at the time of the experiments, I was especially careful to make detailed notes of each case and to look over a large number of living eggs which showed unusual conditions. From the data given in my notes I feel confident that none of the eggs of experiment No. 29 extruded the second polar body, and it is quite unlikely that the spindle shown in Fig. 45, which is one of many similar cases found in the same material, would have later assumed a normal position and thrown off the polar body. Although I have not found all the intermediate stages, numerous cases like Fig. 46 occur in the same series of eggs, and it would seem highly probable that the two nuclei present are the result of the submerged mitosis and represent the egg nucleus and the nucleus of the second polar body. In spite of the absence of indisputable proof, there can be little doubt that the two nuclei fuse to form a cleavage nucleus, since in later stages of the series many eggs are found which contain a single large nucleus accompanied by two asters, while still other eggs

show different stages of the first cleavage mitosis. If my interpretation of these conditions is correct, the case is identical with the rarer type of parthenogenesis described by Brauer ('93) for *Artemia*, where the chromosomes of the second polar body are retained by the egg and give rise to a reticular nucleus, as earlier described by Boveri ('87) in *Ascaris*, which acts like a sperm nucleus and conjugates with the nucleus of the egg. Brauer's observations apparently confirmed Boveri's conception that "Parthénogenesis is the result of fertilization by the second polar body" (*l.c.*, p. 73). Of course, if the fusion of the two nuclei takes place, one would expect to find 24 chromosomes in the equatorial plate of the first cleavage spindle, but unfortunately I have not been able to obtain a favorable section in which the number of chromosomes could be accurately determined, but there are clear indications that it is greater than 12. The difficulty in establishing this point is not surprising, as the material from this experiment, which was preserved for the later stages, was quite limited in amount.

#### *b* Absence of both Polar Bodies

A more frequent abnormality is found in the failure to extrude both polar bodies, yet eggs which show this peculiarity may develop into swimming larvæ. The condition was observed in a number of experiments when after careful search not an egg was seen to give off the polar bodies, but the percentage of developing eggs in such cases was as high as the average. Fig. 47 shows a submerged first maturation mitosis in a late anaphase; the centrosomes are clearly seen to be double and the chromosomes are in the form of dyads and not single rods. It is exceedingly difficult to reconstruct the successive stages in the internal maturation of such eggs, and here again positive proof cannot be furnished. Two resting nuclei are undoubtedly formed, as they occur in many eggs, but I am inclined to believe that these usually fuse at once to form a cleavage nucleus without the occurrence of a second maturation mitosis. Although I have not succeeded in finding a complete series of stages, there is some evidence, however, that in rarer cases the two nuclei just referred to may divide again mitotically, with the resulting formation of four smaller nuclei. In

Fig. 48 is shown an egg in which one of the two nuclei present is accompanied by two minute asters, while a single aster is seen lying close to the other nucleus, evidently in preparation for a second division. Fig. 49 shows four small nuclei which have possibly arisen through two maturation divisions occurring internally. Of course, such a condition as this might be explained as the result of the formation of a tetraster without subsequent cytoplasmic division, but polyasters are entirely absent in the eggs from which these cases are taken, and if this were the correct interpretation, some evidence of the occurrence of multipolar mitosis would certainly be present. It is impossible to determine whether the four nuclei fuse, or not, to form a cleavage nucleus, yet that they do conjugate is indicated by the fact that in later stages of the same material where the cleavage amphaster is present, a careful search fails to disclose accessory nuclei outside in the cytoplasm. In a few sections of the equatorial plate of the first cleavage spindle, found in the same series of eggs, the number of chromosomes is clearly more than twelve, although I have never counted an exact multiple of that number; in Fig. 50 twenty-three chromosomes are present in the section, and this would seem to prove that at least two of the nuclei had united.

In a number of experiments in which it was noticed that none of the eggs extruded polar bodies, besides the cases of internal maturation already described, a still more unusual condition, which may be appropriately referred to in this connection, is apparently present in certain eggs. These are cases in which the first maturation spindle is formed in the ordinary manner, but instead of rotating into a radial position it becomes elongated and placed symmetrically across the center of the egg. These large spindles (Fig. 51), clearly showing the crosses of the tetrads and the double centrosomes, predominate in the eggs of certain experiments, and since later stages of the same material show many eggs divided into two cells, it can hardly be doubted that they give rise to an equal or nearly equal segmentation of the egg, the products of which have, therefore, the value of oöcytes of the second order. Fig. 52 shows an anaphase of such an egg, in which the distinct dyads are seen passing to the poles of the spindle. I am unable to

state, however, whether the next cleavage behaves like the second maturation division or not, but it would be of interest to know if the dyads reappear at that time, as might be expected.

## 7 *Abnormal Mitoses*

It is not my intention to describe in detail the endless variety of abnormalities of mitosis that have been encountered in the study of the parthenogenetic eggs of *Thalassema*. Most of the unusual conditions which I have found are quite similar to those which have been described by other observers, especially by R. Hertwig ('96), Morgan ('96, '99, '00), and Wilson ('01), in the unfertilized eggs of echinoderms after treatment with salt solutions and other agents. It may be well, however, to refer briefly to the more characteristic abnormal forms.

### a *Multipolar Mitoses*

Of these, the formation of polyasters, with resulting multipolar mitoses, was perhaps of the most frequent occurrence and was observed at all stages of development from the first cleavage onward. It has already been stated that the two cleavage asters, when the parthenogenetic eggs develop without abnormalities, appear simultaneously on the nuclear membrane, and give rise to the usual dicentric figure. When three or more asters appear, instead of two, they also seem to arise *in situ*, lying close to the nuclear membrane and at quite a distance from each other. I have never observed a doubling of the centrosomes in these cases of multipolar mitosis, or the least indication of division of the asters. Fig. 53 shows a typical case; the egg nucleus, which is here rather larger than usual, is accompanied by four small asters, showing central bodies, and the membrane is giving way in front of three of them. As the spindles form, the nuclear area is usually drawn out at the points where the asters lie, as seen in one of the cells of Fig. 54, and with the dissolution of the membrane, forms like these give rise to multipolar spindles. Such eggs may divide at once into a corresponding number of blastomeres, as I have frequently observed living eggs in which triasters and tetrasters

were faintly visible to fall into three or four cells, respectively, at the first cleavage. Cytoplasmic division, however, does not by any means always follow a multipolar mitosis which may occur repeatedly in one and the same cell. That this is true is clearly proven by the large number of chromosomes often present in such cells. Fig. 58 shows an abnormal embryo in which one cell has evidently failed to undergo cytoplasmic cleavage and in which a multipolar mitosis is taking place. A similar condition is seen in Fig. 55 which presents a maze of spindles and asters in the unsegmented egg.

Cytasters in the acid treated eggs of *Thalassema* are of rare occurrence, and only occasionally does one find an aster which is not associated with nuclear material. In fact, I have never been able to thoroughly satisfy myself that I have observed a true cytaster, but in cases like Fig. 55, the small asters lying near the periphery of the egg, some of which show a central granule, may possibly be of this nature.

At the close of a multipolar mitosis, the numerous chromosomes are either gathered into a single large nucleus, which is usually polymorphic in character (Fig. 56), or apparently many separate smaller nuclei may be formed which increase in size during the resting period (Fig. 57). Frequently the latter are lobed or constricted, as if dividing amitotically (Fig. 57), and it is also probable that they fuse at times into a single large nuclear area. I am inclined to believe that such eggs rarely segment, as they are especially numerous in dishes in which the developing eggs have reached late blastula and gastrula stages.

#### *b* Giant Bipolar Figures

In more or less abnormal embryos, I have quite often found large cells, in which segmentation had evidently not kept pace with nuclear division, that were characterized by the presence of a single giant spindle bearing an enormous number of chromosomes. Fig. 59 illustrates a case in point. In the cell marked *a*, the spindle is abnormally large, while the equatorial plate is densely packed with small chromosomes which are greatly in excess of the usual number. In cell *b* of the same figure, a similar spindle, but

smaller and containing fewer chromosomes, has been cut transversely in the middle. It is difficult to determine how figures like these have arisen, but since the number of chromosomes is greatly increased, nuclear division has undoubtedly occurred repeatedly without accompanying cleavage of the cytoplasm. Their origin could be accounted for by supposing that, after several nuclear divisions have taken place, probably through multipolar mitosis but without cleavage of the cytoplasm, all of the chromosomes have been gathered into a single large nucleus which, at the next mitosis, is converted into a bipolar figure by the appearance of only two asters in connection with it.

#### c Monasters

Mention should be made of another common type of abnormal forms, the striking single radiate systems or monasters which have been described by previous observers, notably Hertwig ('96), Morgan ('00) and Wilson ('01).

In *Thalassema* the monasters are only found in unsegmented eggs, the closely set rays forming a beautiful corona around the nucleus. I have never observed the monaster to resolve itself into a bipolar figure, nor to produce a segmentation, but the same alternating phases of activity, involving the rhythmic disappearance and reappearance of the rays and successive division of the chromosomes, as have been described by Wilson ('01, p. 546) for *Toxopneustes*, also occur in the monasters of *Thalassema*. These periodic changes are undoubtedly comparable, as Wilson maintains, with the progressive transformations of the nucleus in dividing eggs. I have frequently been able to observe in the living egg the periodic changes in the rays of the monasters, although they cannot be seen very distinctly. Sections show that the chromosomes usually lie at the center of the aster in a clear, hyaline area, from the border of which the rays diverge (Fig. 60). In a few cases, some of the chromosomes are found scattered among the rays, although this condition does not seem to be the common one, as it is in *Toxopneustes*, and in none of the monasters in my material have I found such figures as Wilson has described, where the center is formed by a spongy centrosome from which the rays radiate (*l.c.*, Figs. 40, 41).

In Fig. 60 the chromosomes are very numerous, and several divisions must have occurred to produce this condition. Some of my sections (Fig. 61) show cases similar to those of Wilson in which the chromosomes are actually found to be splitting longitudinally, and there can be no doubt, when the number of chromosomes in the monaster is greater than in the egg nucleus, that division of the chromosomes has occurred at each active phase of the cycle. Not only are the chromosomes found dividing among the rays, but more frequently the longitudinal splitting takes place in the central clear area of the aster, as seen in the last figure. Here one is lying on the rays and is evidently in the act of dividing, while the rest, grouped in the center, have either split to form double rods or are taken in some stage of the process. By counting the chromosomes in this and the other sections of the same egg, it was found that about 24 double rods were present, so that in all probability the division which is occurring at this time is the second one in the recurring transformations of the monaster. Fig. 62 shows a monaster in which the rays do not quite reach the central area; it is probable that the egg was killed just as the radiations were beginning to reappear.

## VII GENERAL CONSIDERATIONS

### I *Differentiation Without Cell Division*

It has been shown in the preceding pages that certain solutions of acids furnish an efficient stimulus for the development of unfertilized eggs of *Thalassema* into embryos and larvæ, and, furthermore, that this development in favorable experiments closely approximates, if it is not identical with, the normal processes of differentiation leading up to the formation of the swimming trochophore.

Previous experimenters on artificial parthenogenesis of annelids have obtained with the methods which they have employed only abortive attempts at development, and their embryos and larvæ have widely departed from the normal in almost all respects. Especially aberrant is the case of *Chætopterus* (Loeb, '01, Lillie '02) in which the unfertilized eggs may be caused to undergo certain cytoplasmic differentiations in the entire absence of cell divi-



sion and produce ciliated structures faintly simulating the appearance of trochophore larvæ. My observations on *Thalassema*, however, bring the annelids into closer accord with the results which have been obtained with echinoderms, especially with the starfish, where parthenogenetic development has been shown to be far more normal in character than in other groups of animals experimented with.

The differences between the parthenogenetic embryos of *Thalassema* and the structures which have been obtained from the unfertilized eggs of other annelids, notably *Chætopterus*, are most marked and involve important considerations. Differentiation without cell division has never been observed in *Thalassema*, but on the contrary progressive differentiation of the embryo in this case depends upon cell division at every stage.

The formation of the differentiated masses of ciliated protoplasm which he observed in *Chætopterus*, Lillie insists, must be interpreted as just "as truly a process of development as the formation of the trochophore" ('02, p. 493). The eggs, without dividing into cells "pass through well defined phases of differentiation, the yolk accumulating in a dense mass in the interior, and the peripheral cytoplasm becoming vacuolated and ciliated. The ciliated ectoplasm and the yolk laden endoplasm are analogous to the ectoderm and endoderm of the trochophore, and the phases of differentiation resemble some of the normal processes, though the resulting object can by no stretch of the term be properly called a trochophore" (*l.c.*, p. 477). Furthermore, "in some cases it is even possible to homologize the regions of these unsegmented ciliated eggs with the regions of the trochophore" (p. 495). The process of cell division, as such, Lillie concludes, "is necessary neither to growth, differentiation, nor the earliest correlations; but it is accessory in Metazoa, to all three as a localizing factor, often from the earliest stages" (p. 494).

These results are in sharp contrast with my observations on *Thalassema*, as it has been shown that the processes of differentiation, if they do not depend upon cell division, nevertheless, do not occur in its absence. In this form, if the egg remains unsegmented, no differentiation of the cytoplasm takes place and a ciliated em-

bryo is never produced. I have never found a single instance of the occurrence of the pseudo-trochophore described by Lillie and others. It would seem true, therefore, that in the development of *Thalassema* at all events, *cell division is something more than a mere "localizing factor;" it is rather, on the contrary, fundamental and essential to all processes of differentiation and correlation.*

Not only does the conclusion just expressed seem to be the correct one, but it is also undoubtedly true that the more closely the course of progressive cellular differentiation follows in the path of the normal processes of development, the more nearly normal is the resulting trochophore, both structurally and functionally. Abnormalities in cleavage at any stage seem to permanently disturb the organization of the embryo, while the resulting defects and deficiencies do not appear to be made good later on.

It is of interest to remark in this connection, that while differentiation of the egg does not occur in the absence of cell division, cytoplasmic cleavage in *Thalassema* does not appear to take place without preceding division of the nucleus. From the most careful examination of my sections, I have found no evidence that the cytoplasm segments if unassociated with mitotic phenomena involve a distribution of chromatin. This fact is not in accord with the observations which have been frequently made on other eggs, and may possibly be correlated to a certain extent with the absence, or, at any rate, the very rare occurrence of cytasters in the eggs of my experiments.

Finally, it may be remarked that, since the parthenogenetic trochophores of *Thalassema* possess a highly normal organization with a differentiated digestive tract, etc., a physiologically self-sustaining organism would seem to be a possibility in the parthenogenetic development of this worm, and that success in rearing the larvæ to maturity must depend upon the finding of satisfactory means of nurture for tiding the animals over the critical period. This accomplishment undoubtedly lies within the bounds of experimental investigation.

2 *Origin of the Cleavage Centrosomes*

The formation *de novo* of the centrosome, at first rejected by Boveri ('01) but later accepted by him ('02, p. 40) on the evidence furnished by Wilson's experiments ('01), has been recently attacked by Petrunkevitch ('04), who has attempted to defend the continuity of the centrosome while gratuitously assuming that the ovocenter, although invisible and undiscoverable after maturation, persists in the parthenogenetic egg and later gives rise to the cleavage centrosomes. His contention that the centrosomes of the multiple-asters are not new formations but arise by division of the primary egg center, as do all asters containing central bodies, and, furthermore, that the cytasters of egg fragments do not possess central bodies, has been adequately criticised by Wilson ('04) and shown to be utterly unsupported by evidence. The observations of both Wilson ('01) and Yatsu ('04, '05), that asters containing centrosomes can be artificially induced in egg fragments, in which there is no possibility of the presence of an egg center, completely sets at rest the question of their formation *de novo*, and in the light of these facts the probability of a similar origin of the cleavage centrosomes in parthenogenetic eggs seems to me to be very great. Since it has been experimentally proven that centrosomes may be induced as new formations, it is very difficult for me to conceive of the centrosome and its associated radiations as anything more than an expression in cell substances of forces or activities tending to produce cell division; that is, as an effect rather than a cause—an opinion, I believe, which is becoming more generally prevalent. With the destruction of the older conception of the centrosome as a persistent cell organ, any attempt to rescue even a shred of the former theory, in maintaining a physiological unity for the centrosome as an active stimulating agent in cell division, must, it seems to me, be futile.

The independent origin of the cleavage centrosomes in the parthenogenetic egg of *Thalassema* suggests the possibility that in the normally fertilized egg they may not be derived from the sperm center, but that they, too, arise as new formations in the cytoplasm. Since Griffin's work on this egg, *Thalassema* has

generally been regarded as furnishing strong evidence of the persistence of the cleavage centrosomes and their direct continuity with the sperm center. The doubt expressed above has led me to examine with great care the entire period in the normally fertilized egg from the first appearance of the sperm aster to the establishment of the cleavage amphiaster, and the results which I have obtained from these observations are not in accord with Griffin's description. He was confident that he had traced the sperm centrosome continuously into the cleavage centers, and stated his conclusion as follows: "In *Thalassema*, 'the pause' is of short duration, and while the asters are a trifle less distinct, they nevertheless show clearly throughout, and the persistence of their focal centrosome is easily demonstrated. \*\*\* In most instances the presence of the centrosomes can be made out with comparatively little difficulty. With the commencing fusion of the nuclei, the centrosomes take up a polar position, and immediately become the centers of renewed activity, for many additional rays commence to start up about them. From the above it is quite evident that *the centrosomes persist entire throughout the whole critical stage where, in so many forms, they have been lost sight of*" ('99, p. 598).

The material upon which I have made my observations was preserved at intervals of one minute throughout the entire period in question, and the sections made from it are as nearly perfect as sections can be. Contrary to Griffin's observations, I have found a stage, just before the fusion of the pronuclei, when the rays of the asters become exceedingly faint, if they do not disappear entirely, and the most careful search fails to reveal the presence of centrosomes. A little later, upon the reappearance of the rays, the centrosomes of the cleavage amphiaster can be demonstrated. I am convinced, therefore, that a critical stage exists in *Thalassema*, as in many other forms, and at this time the continuity of the sperm centrosomes cannot be followed. Kostanecki ('06), in a very recent paper, has undertaken an elaborate attempt to prove the universality of the origin of the cleavage centrosomes (centrioles) from the centriole of the spermatozoön, and has convinced himself "das im befruchteten Ei sämtlicher Metazoen die Centriolen der ersten Furchungsspindel die direkten Abkömm-

linge des von Spermatozoon eingeführten Centriols sind. Die von dieser Regel statuierten Ausnahmen erweisen sich bei genauerer Prüfung als unhaltbar" (p. 429). In order to arrive at this conclusion, he rejects all conflicting observations of others, in many cases on entirely insufficient grounds. Griffin's account for *Thalassema* is emphasized by Kostanecki as furnishing strong support for his position, yet an examination of the same egg has led me to seriously doubt the genetic continuity even in this case between the sperm center and the centers of cleavage.

In the light of my observations, therefore, it is difficult for me to avoid the suspicion, at all events, that in the normally fertilized eggs of *Thalassema*, as in those which develop parthenogenetically, the cleavage centrosomes arise *de novo* and are caused to appear in the egg cytoplasm upon renewal of those activities which lead to the division of the cell.

### 3 *Numerical Relations of the Chromosomes*

It has been seen that the number of chromosomes (24) characteristic of the fertilized egg of *Thalassema* is not restored during parthenogenetic development, but that the reduced number is retained throughout and has been repeatedly counted even in late blastula and gastrula stages. This result is in accordance with the observations of several others, who have determined the persistence of the changed numerical relations of the chromosomes when their number has been altered, as in the fertilization of enucleated egg fragments and in artificial parthenogenesis, or under other experimental conditions. This fact has been shown to be true by Morgan ('95), Boveri ('95, '05), Wilson ('01) and Stevens ('02). The parthenogenetic eggs of *Thalassema*, therefore, bear out the contention, so strongly made by Boveri, that a restitution of the normal number of chromosomes does not take place when the number has been either increased or diminished by unusual conditions. The fact, however, is opposed to the results which Delage ('99, '01) has derived from his experiments on merogony and artificial parthenogenesis in the sea-urchin and which have led him to maintain that the normal number of chromosomes is a specific character and is restored when it has been disturbed. Boveri

('02b, '05), on the other hand, has not only proved that Delage erroneously determined the number of chromosomes in the fertilized eggs of *Strongylocentrotus* to be 18, whereas it is 36, a blunder which entirely vitiates his conclusion with respect to the chromatin relations of his parthenogenetic sea-urchin larvæ, but he has also shown it to be highly probable that, in his experiments on merogony, Delage was dealing with abnormal conditions which might easily have led him into error regarding the number of chromosomes present in his embryos. Morgan ('95), moreover, several years before Delage's work on merogony, had found the reduced number of chromosomes persisting in early cleavage stages of fertilized enucleated fragments of the sea-urchin's egg, an observation, however, to which Delage makes no reference.

In a later paper on artificial parthenogenesis of *Asterias glacialis*, Delage ('02c) states that a preliminary examination of his preparations has led him to believe that the number of chromosomes in the morula and blastula is the same as in embryos arising from fertilized eggs, that is, 18, but as he offers no evidence in support of the statement, it cannot be accepted without further investigation.

Although it is safe to conclude that the initial number of chromosomes persists, it must be borne in mind, as Boveri, Wilson, and others have pointed out, that abnormal conditions may intervene to disturb these relations and lead to a multiplication of chromosomes in a single cell, as may be seen, for example, in the case of monasters and other pathological mitoses where longitudinal splitting of the chromosomes may occur without accompanying cleavage of the cytoplasm, as well as in eggs that have been entered by supernumerary spermatozoa.

The unusual conditions which I have described in the maturation of the unfertilized eggs of *Thalassema*, whereby the cleavage nucleus probably receives, not only the chromatin of the egg nucleus, but that of one or both of the polar bodies as well, presumably lead to an increase in the number of chromosomes throughout subsequent mitoses. This may be also true of *Asterias glacialis*, since, according to Delage, the eggs of the starfish, like those of *Thalassema*, may at times fail to extrude one or both polar bodies. I have not been able thus far to prove that later

embryos arising from these abnormal eggs possess the additional chromatin, although I have found indications of such a condition, but I shall attempt to investigate this point at a future time when adequate material may be at hand for the purpose.

#### SUMMARY

1 The unfertilized eggs of *Thalassema mellita* may be induced to develop parthenogenetically into actively swimming trochophores by immerison for a few minutes in dilute solutions of acids, both inorganic and organic.

2 After transfer from the acid solutions into normal sea-water, the egg throws off a typical fertilization membrane, the germinal vesicle breaks down, and maturation and cleavage follow. In successful experiments, which were the rule, from 50 to 60 per cent of the eggs developed into swimming larvæ that could scarcely be distinguished from normal trochophores of a corresponding stage.

3 The parthenogenetic development, in the majority of cases, involves a strictly normal maturation, a normal cleavage, at least in the early stages, and the usual processes of differentiation that occur after fertilization by sperm.

4 Gastrulation takes place in the normal manner, and the parthenogenetic larva possesses a digestive tract, differentiated into mouth, œsophagus, stomach and intestine, and the prototroch and apical plate bearing the normal arrangement of cilia.

5 After maturation, the egg center disappears, and the cleavage centrosomes arise *de novo*, probably without division of a single primary center, and, when first seen, lie on opposite sides of the egg nucleus which becomes the first cleavage nucleus.

6 Cell division occurs mitotically throughout development, and division of the nucleus is usually accompanied by cytoplasmic cleavage.

7 The number of chromosomes characteristic of the fertilized egg is not restored, but the reduced number (12) is retained and has been counted repeatedly, even in late stages.

8 The rate of division is not as rapid, nor as regular as in normal segmentation, and the parthenogenetic larvæ, although swimming

vigorously at the bottom of the dish, do not rise to the surface of the water.

9 After exposure of the eggs to acid solutions, the polar bodies may continue to divide mitotically and form a morula-like cluster of minute cells, thus exhibiting an attempt at parthenogenetic development.

10 In some experiments, the eggs extruded only one polar body and in others neither polar body was formed. In such cases, either one or both maturation mitoses take place inside the egg, with the resulting formation of resting nuclei which probably fuse to form a cleavage nucleus. In still other cases, there is evidence for believing that the first maturation spindle may directly become the first cleavage spindle, across which the egg divides into equal or subequal cells. The numerical relations of the chromosomes in these cases have not been definitely determined. Eggs exhibiting these abnormalities of maturation give rise to larvæ indistinguishable from eggs which mature normally.

11 An endless variety of abnormal cleavages, similar to those described by others, have been observed. Such cleavages lead to the formation of ciliated, cellular structures which, however, depart more or less widely from normal embryos.

12 Abnormalities of mitosis, as polyasters and monasters, are not infrequent, and when nuclear division is not followed by cleavage of the cytoplasm, chromosomes in excess of the usual number (12) may be found in a single cell.

13 Cytasters are either absent or exceedingly rare, and cytoplasmic cleavage without preceding nuclear division has not been observed.

14 Amœboid movements of the egg are rare, and when they occur, are not extensive; "fusion phenomena" are lacking.

15 Cell division would seem to be a fundamental and essential factor in differentiation, since in no instance was a differentiated, ciliated structure observed which was unsegmented; the parthenogenetic pseudo-trochophores, which have been described for *Chæptopterus* and other annelids, are entirely absent.



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## PLATE I

All of the figures of this and the succeeding plates were drawn from parthenogenetic material, with the aid of the camera. The drawings were made under a magnification of 1000 diameters, except Figs. 5-14, as well as Fig. 16, in which the magnification was 700 diameters. The dotted line, which is drawn in many of the drawings surrounding the nucleus or mitotic figure, encloses the yolk-free area.

All figures are from living material, except 2 and 3

Fig. 1 Living egg, showing delicate protoplasmic threads extending from the surface of the egg to the membrane, and also multiple polar bodies.

Fig. 2 Section of full-grown oöcyte immediately after treatment with acid but before breaking down of germinal vesicle; the membrane, which was present, is not represented.

Fig. 3 Section of egg in which the germinal vesicle has migrated to animal-pole after acid-treatment.

Fig. 4 Living egg showing three polar bodies; the figure was drawn immediately after the division of the first polar body.

Figs. 5-8 Two, four, eight and sixteen-cell stages drawn from living eggs.

Fig. 9 Young HCl trochophore, 15 hours old, showing apical flagella, cilia of prototroch and blastopore *bl*.

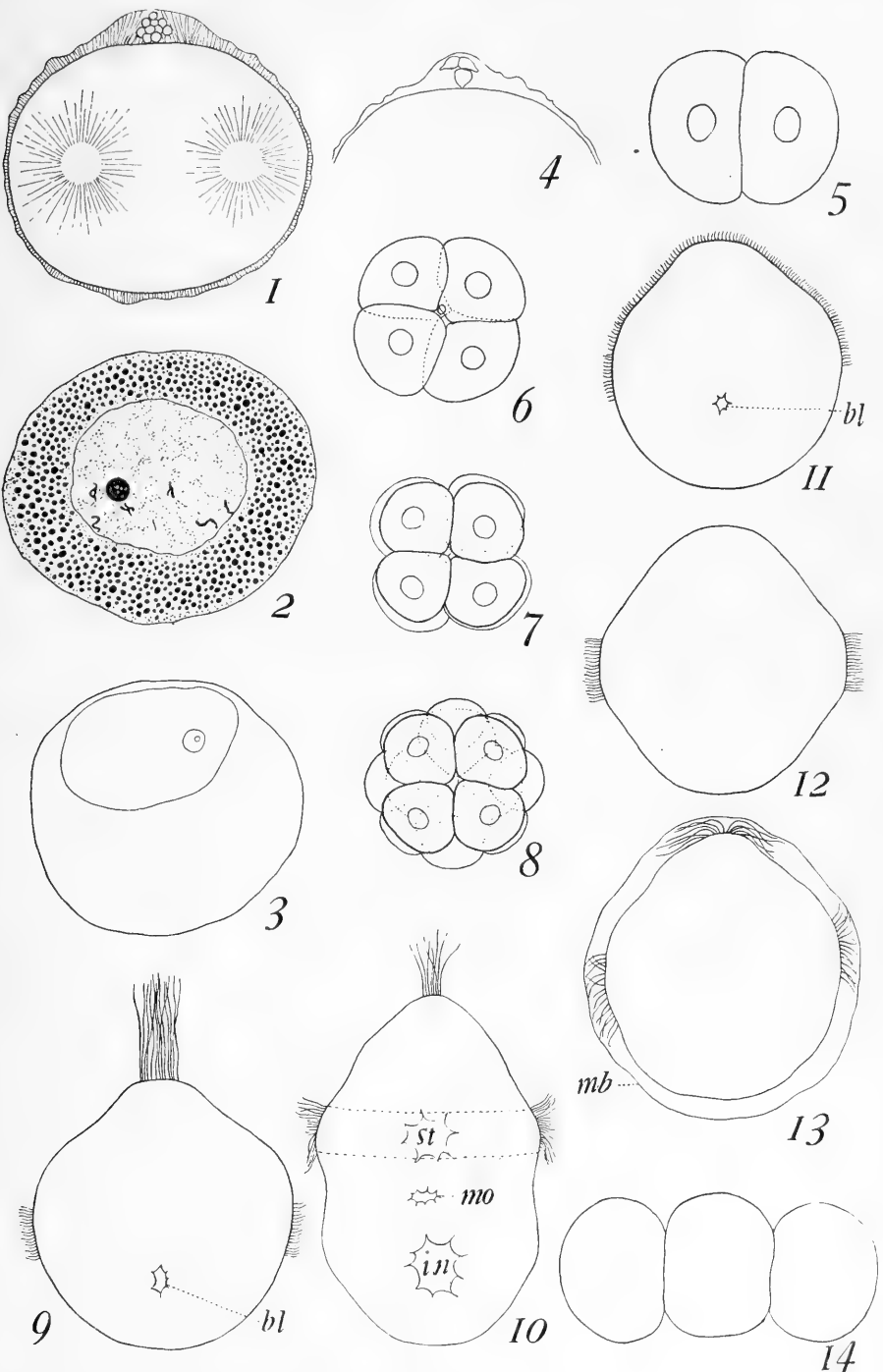
Fig. 10 HNO<sub>3</sub> trochophore, 36 hours old, showing the larger cilia on upper and lower border of prototroch and the mouth *mo*; the cavities of stomach *st*, and intestine *in*, are seen at a deeper level.

Figs. 11-12 Young trochophores, showing abnormal ciliation; in the former, short cilia cover the entire pre-trochal region, while the latter lacks the apical flagella.

Fig. 13 Abnormal embryo, in which the cilia have failed to puncture the membrane *mb*, and are pressed down by latter.

Fig. 14 Abnormal 3-cell stage, formed at second cleavage.

GEORGE LEFEVRE



## PLATE II

All figures are from sections, except 15 and 16

Fig. 15 Optical section of living, abnormal blastula which was cylindrical in form and open at both ends.

Fig. 16 Egg showing amœboid activity after treatment with acidulated water.

Fig. 17 Simultaneous appearance of the two asters, with centrioles, on wall of germinal vesicle.

Fig. 18 Breaking down of germinal vesicle and formation of first maturation spindle; the centrosomes are double and the fibers passing to chromosomes are thicker than rest.

Figs. 19-20 First maturation spindles, fully formed, the latter in its definitive position; the chromosomes (tetrads) are in the form of crosses, in some of which the perpendicular split may be seen.

Fig. 21 Formation of second maturation spindle; dyads are seen in both the egg and the first polar body; two centrioles with a few delicate rays are present in latter.

Fig. 22 Second polar spindle in definitive position; the dyads are in the equatorial plane with the longer axis lying transversely to the spindle.

Fig. 23 Anaphase of second polar mitosis; the inner centrosome is double. The first polar body shows mitotic activity.

Fig. 24 Close of second maturation mitosis, showing 12 single chromosomes in egg, as well as several similar chromosomes in the second polar body.

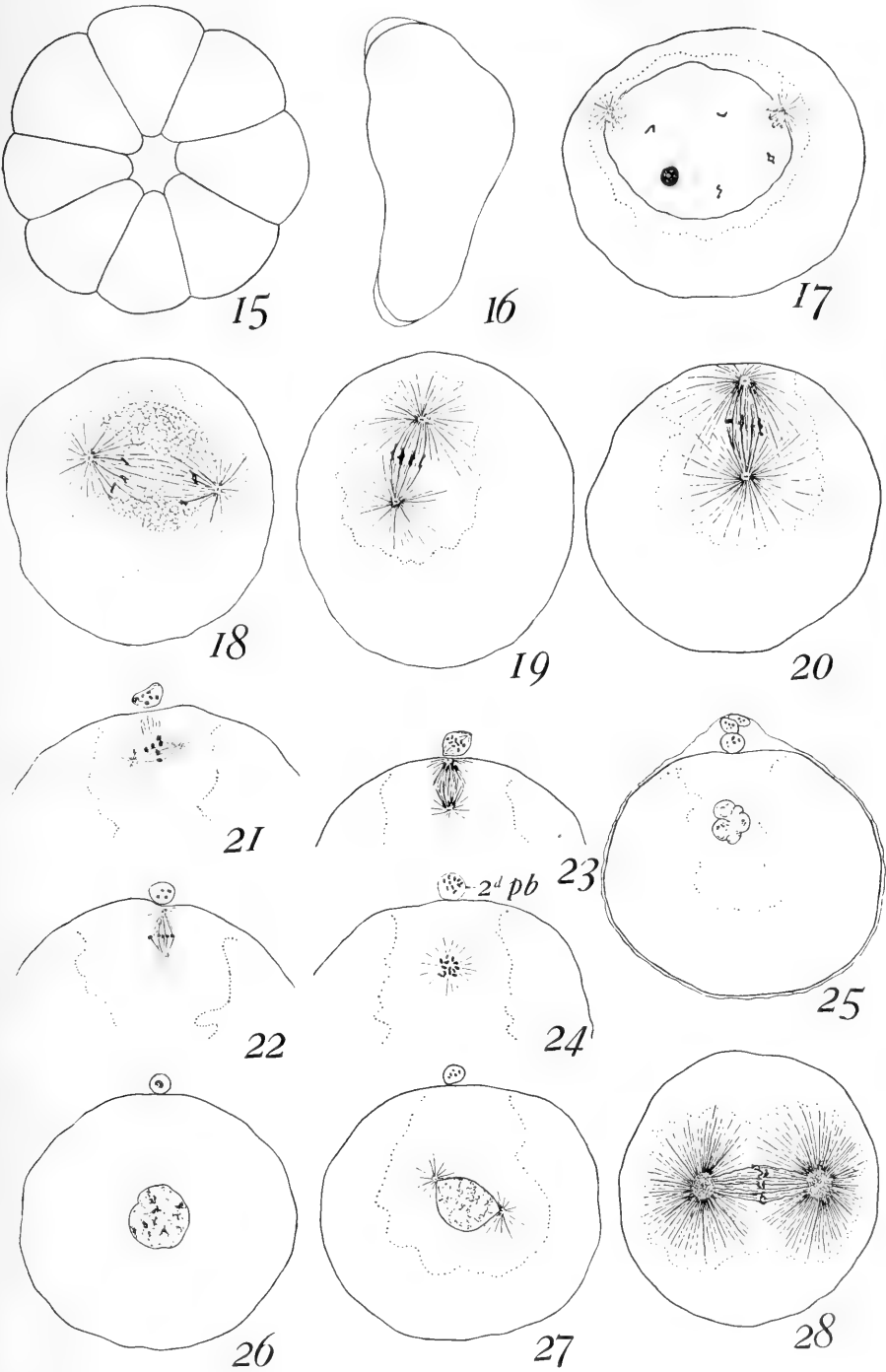
Fig. 25 Chromosomal vesicles partially fused to form the egg nucleus; centrosomes and rays have totally disappeared.

Fig. 26 Egg nucleus during the "pause."

Fig. 27 Simultaneous appearance of the two cleavage asters with their centrosomes on opposite sides of the egg nucleus.

Fig. 28 Fully formed, first cleavage figure; the large, reticulated centrospheres contain divided centrosomes, and the spireme is segmenting into chromosomes.

GEORGE LEFEVRE





### PLATE III

All figures are from sections

Fig. 29 Transverse section of equatorial plate of first cleavage mitosis, showing 12 chromosomes, the reduced number.

Fig. 30 Telophase of first cleavage, showing chromosomal vesicles and daughter amphiasters.

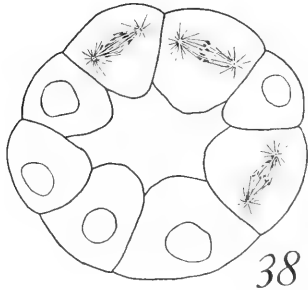
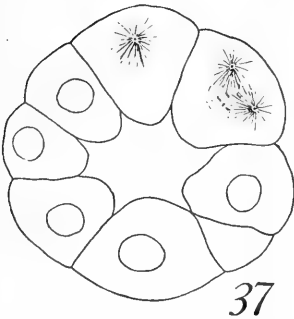
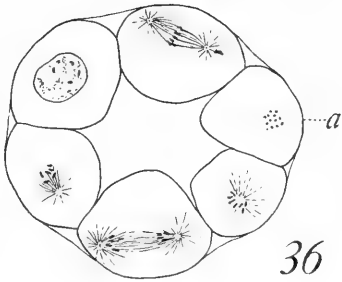
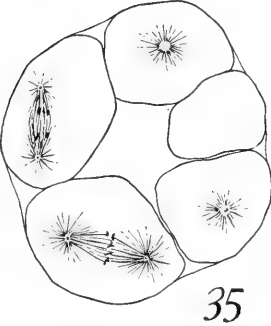
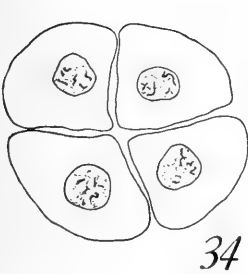
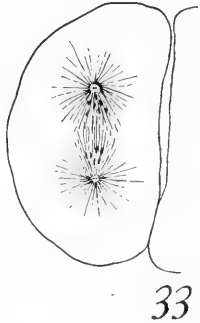
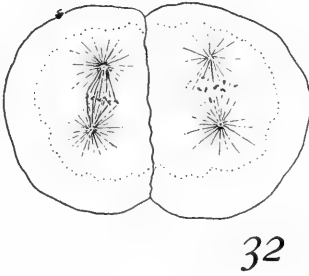
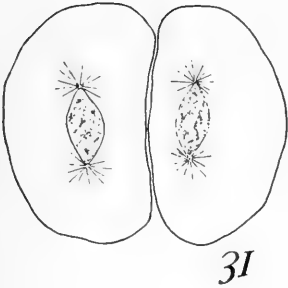
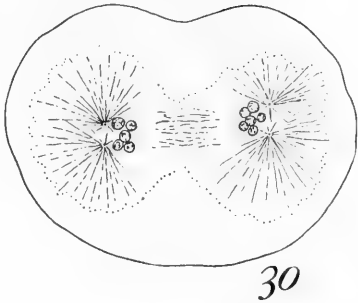
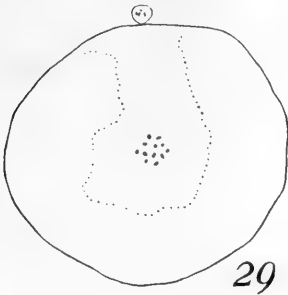
Figs. 31-32 Prophases of second cleavage.

Fig. 33 Anaphase of second cleavage, showing divided centrosomes.

Fig. 34 Four-cell stage, resting condition.

Fig. 35 Eight cell stage in section.

Figs. 36-38 Sections of young blastulæ, showing cells in various stages of mitosis. In Fig. 36, a transverse section of the equatorial plate with its 12 chromosomes is shown at *a*. Divided centrosomes are seen in some of the mitotic figures.



#### PLATE IV

All figures are from sections\*

Fig. 39 Young blastula with abnormally large blastocœl.

Fig. 40 Section of an early gastrulation stage, showing the flattening of the lower pole of the embryo and the large entoblastic cells just before sinking in. Several rudimentary cells *rc* are seen in the cleavage cavity; one lies inside an endodermal cell. A polar body *pb*, showing mitotic activity, is in the blastocœl.

Fig. 41 Section of a young gastrula, showing a mass of entomeres within the cleavage cavity; the cell marked *oes* is probably an œsophagoblast. At *a* the 12 chromosomes of an equatorial plate are seen. Rudimentary cells are shown at *rc*.

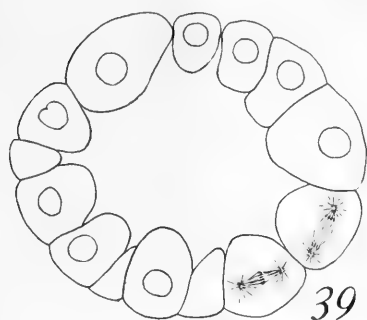
Fig. 42 Later gastrula, showing entoblastic mass with beginning cavity *en*. The stippled cells in this and the next two figures are cells of the larval mesenchyme.

Fig. 43 Later stage, showing a larger enteric cavity and also the ectodermal, œsophageal invagination; the cells marked *oes* are probably œsophagoblasts.

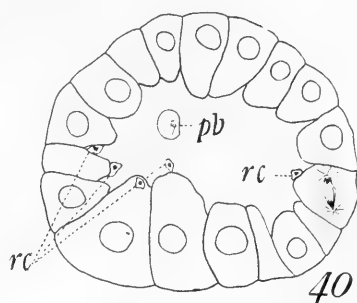
Fig. 44 Horizontal section of a CO<sub>2</sub> trochophore, 31 hours old. The enteric cavity is now divided by a septum into stomach *st* and intestine *in*. The prototroch and apical plate are also seen.

Fig. 45 Egg showing submerged second maturation spindle, with chromosomes at poles. The first polar body is preparing to divide.

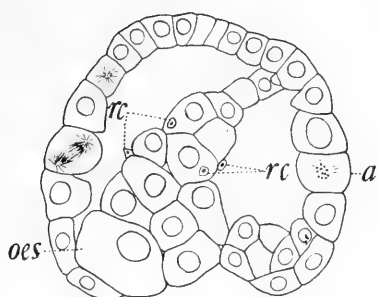
Fig. 46 Egg showing two resting nuclei, probably the result of the mitosis seen in last figure. The mitosis of the first polar body is in anaphase.



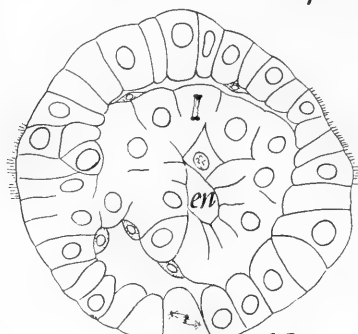
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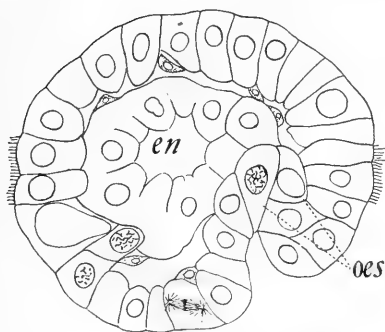
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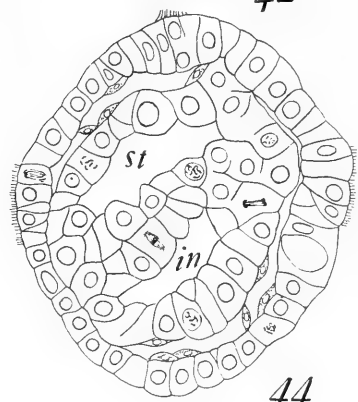
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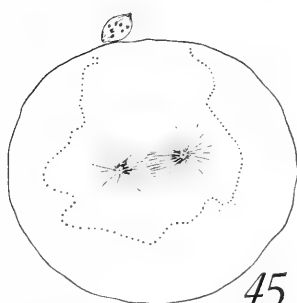
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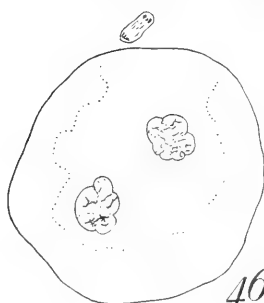
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## PLATE V

All figures are from sections

Fig. 47 Submerged first maturation spindle, showing divided centrosomes and dyads passing to the poles.

Fig. 48 The two nuclei, which have probably resulted from the mitosis seen in the last figure, are preparing for a second mitosis.

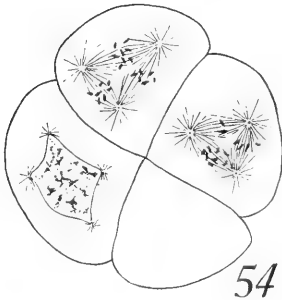
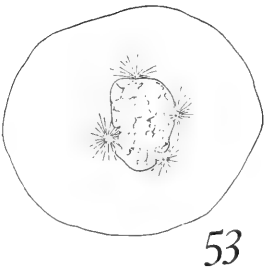
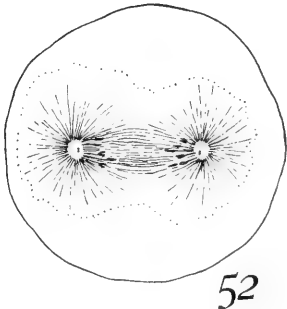
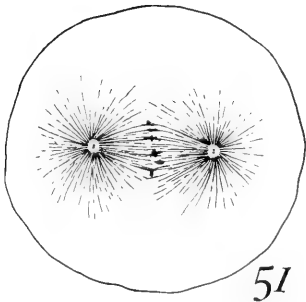
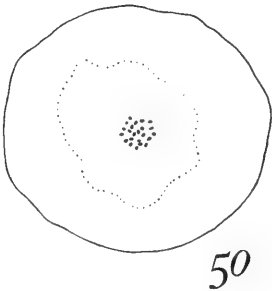
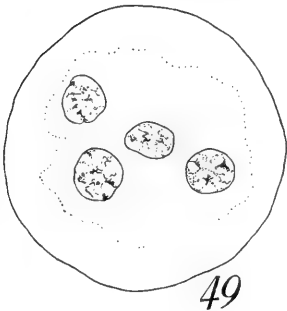
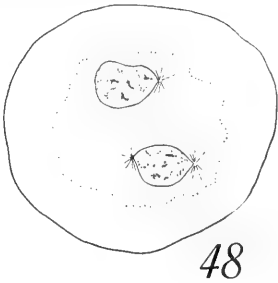
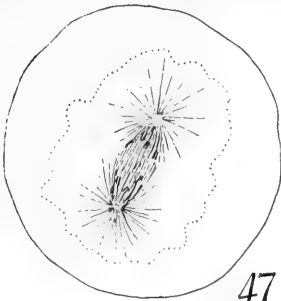
Fig. 49 Probably later stage of the same; the four nuclei may have arisen from a second submerged mitosis.

Fig. 50 Transverse section of equatorial plate of first cleavage spindle, containing 23 chromosomes; the increased number of chromosomes is doubtless due to the fusion of two submerged maturation nuclei to form the cleavage nucleus.

Figs. 51, 52 Enlarged first maturation figures, which probably behave as cleavage figures and lead to an equal division of the egg. In the anaphase shown in Fig. 52, the chromosomes are double and have the typical appearance of dyads.

Fig. 53 Prophase of multipolar mitosis in undivided egg.

Fig. 54 Abnormal four-cell stage, showing multipolar mitoses in three cells.



## PLATE VI

All figures are from sections

Fig. 55 Undivided egg containing multiple asters and spindles; the separate asters near the periphery are probably cytasters, some of which show centrioles.

Fig. 56 Single, large nucleus containing numerous chromosomes, which have probably been gathered together after a multipolar mitosis.

Fig. 57 Undivided egg containing multiple nuclei, which have probably arisen through multipolar mitoses.

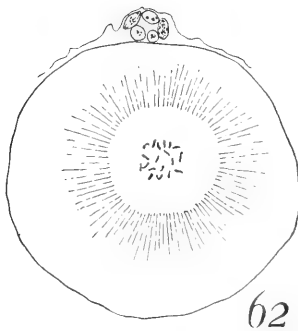
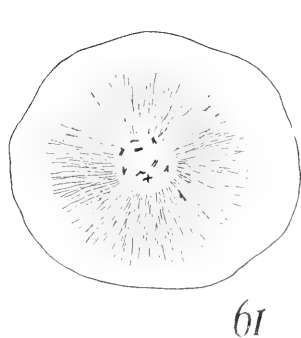
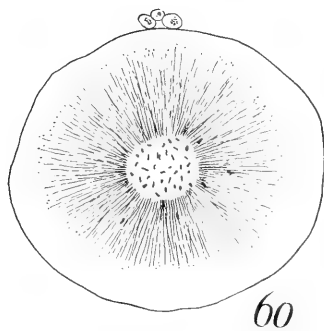
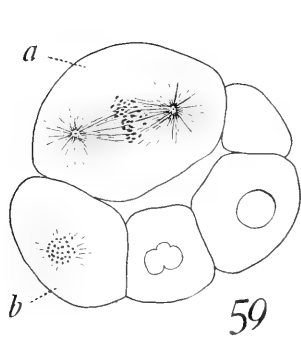
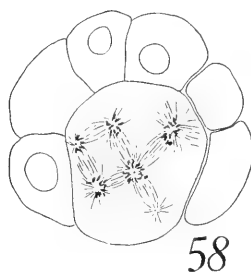
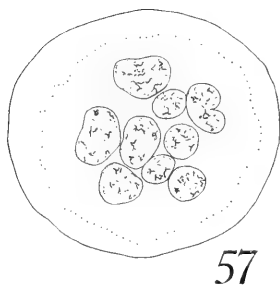
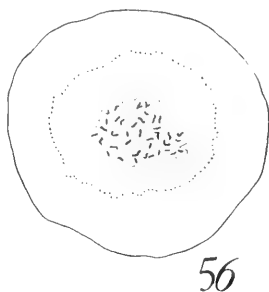
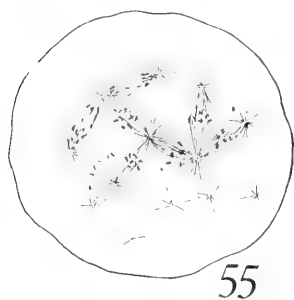
Fig. 58 Abnormal embryo, showing one large cell with multiple spindles and asters.

Fig. 59 Abnormal embryo, showing two giant spindles and their multiple chromosomes.

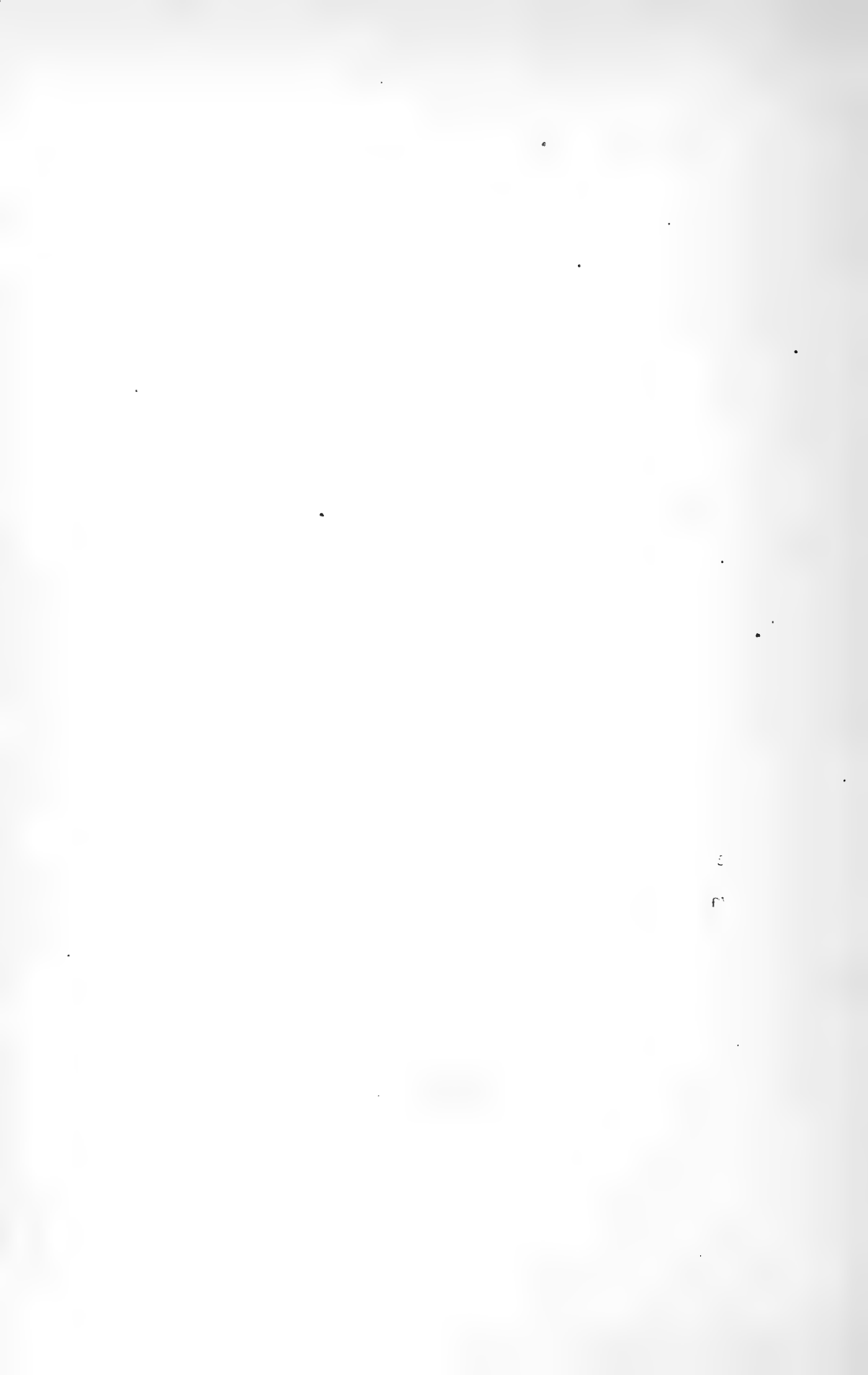
Fig. 60. Monaster in undivided egg; numerous chromosomes are present in the central, clear area, while a few are scattered among the rays.

Fig. 61 Monaster in unsegmented egg; the chromosomes in the central area are dividing or have divided, while one lying outside is also in the act of dividing.

Fig. 62 Monaster in unsegmented egg; this is probably a prophase of a period of activity which will culminate in another division of the chromosomes.







# CONCERNING THE THEORY OF TROPISMS

BY

JACQUES LOEB

About twenty years ago<sup>1</sup> I began a physico-chemical analysis of the behavior of lower animals which had heretofore been explained in the anthropomorphic way characteristic of archaic science. My main efforts were directed toward the analysis of the rôle which light and gravitation play in the reactions of animals. I showed first, that the orientation and the direction of the progressive motion of certain animals can be controlled unequivocally by the direction of the rays emanating from a source of light, and I showed, moreover, that this type of reaction is, as far as we can judge, in every point identical with the heliotropic reaction of plants. A few years later I showed that there exists another group of animal reactions to light, which is not covered by the theory of tropisms, but which depends upon the rapidity of the change of the intensity of the light.<sup>2</sup> This latter type of reaction I designated as *Unterschiedsempfindlichkeit*. Those who are familiar with the terminology of the physicist will most readily understand the difference between the two types of reaction if I state that heliotropism depends upon the value  $f(i)$ , where  $i$  is the intensity of light, while in *Unterschiedsempfindlichkeit* the reaction depends upon the value of  $\frac{di}{dt}$  where  $t$  is the time. Both forms of reaction may occur in the same animal (*e. g.*, *Spirographis*), but this is neither necessary nor the rule.

<sup>1</sup>My first two papers on animal heliotropism and geotropism appeared in January, 1888, and not, as is often stated, in 1890 (*Sitzungsber. der Würzburger Phys.-med., Gesellschaft*, 1888).

<sup>2</sup>I refer the reader to the following papers: Pflüger's Archiv, vol. 54, p. 100, 1893. (Studies in General Physiology, vol. 2, p. 286.) Pflüger's Archiv, vol. 56, p. 247, 1894. (Studies, vol. 2, p. 345.) Pflüger's Archiv, vol. 66, p. 439, 1897. Comparative Physiology of the Brain. Dynamics of Living Matter, pp. 135-137.

The reader will readily notice that I did not attempt to show that *all* animal reactions are of the type of tropisms; on the contrary, I was, as far as I am aware, the first to point out that there exists a type of reactions which are as different from tropisms as are quantities of the dimension of an acceleration from those of the dimension of a velocity. My aim was to analyze the behavior of animals from a physico-chemical point of view and substitute the methods of modern science for the anthropomorphisms of the metaphysician. In this attempt it made no difference to me whether the elementary components of the complex "Animal Behavior" were found to be of the type  $f(i)$ , (e. g., tropisms) or of the type  $f\left(\frac{di}{dt}\right)$  (e. g., Unterschiedsempfindlichkeit) or of any other definite function. Moreover, I laid emphasis on the fact that it is necessary to control the animal reactions before explaining them, as only the control of the reactions offers a sufficient test for the correctness of our analysis. From this point of view I stated that for the control of heliotropic reactions the intensity of the light may remain constant during the experiment, while for the control of the reactions of the type of Unterschiedsempfindlichkeit the intensity of the light must change with a certain rapidity during the time of the experiment.

Whether it is due to mere carelessness or some other cause, a number of American authors have disregarded this discrimination, making their readers believe that the cases of Unterschiedsempfindlichkeit are represented by me as examples of tropisms and then showing that the facts do not coincide with what they state to be my theory of tropisms. I will give a definite instance of this procedure.

In 1893 I described a case of Unterschiedsempfindlichkeit in a tubicolous worm at Naples, *Serpula uncinata*, showing that when the rapidity of the decrease of the intensity of light reaches a certain value a contraction of this worm is caused, while an equally rapid increase in the intensity of light causes no such reaction. In later writings I especially singled out this reaction to illustrate the typical difference between Unterschiedsempfindlichkeit and

tropism. To quote from a paper I published in 1897.<sup>3</sup> "I noticed in the course of my investigation that besides the heliotropic effects there exists a second kind of mechanical effects of light which is determined by the rapidity of the change of the intensity of light and which I designated as *Unterschiedsempfindlichkeit* \* \* \* I found this type of reaction in tubicolous annelids-*e. g.*, *Serpula uncinata*. The gills of these animals protrude from the tube. If we move our hand between the animal and the source of light it rapidly withdraws into its tube as soon as the shadow strikes it. In order to find out whether positive and negative changes in the intensity of light have the same effect I made the following experiment: A glass aquarium which was covered with a glass plate was placed on a table, about two meters from the window. When I rapidly closed the shutters the worms rapidly withdrew into their tubes, as a snail would upon a sudden touch. The shutters did not close tightly and it was sufficiently light in the room to observe the animals. If one waited a little the animals again stretched their gills out of the tube. When now the shutters were rapidly opened, no reaction on the part of the animals occurred. When they were inside the tube the opening of the shutters did not cause them to reappear. It is, therefore, only the decrease in intensity which acts as a stimulus upon the animals."

In the same paper I pointed out that in the physiological effect of the galvanic current physiologists discriminate between the effects dependent upon a constant current and the effects which depend upon the rapidity of the changes in the intensity of a current; and I showed that these differences correspond to the differences between a tropism and *Unterschiedsempfindlichkeit*.

Since inductive effects depend upon the value of  $\frac{di}{dt}$  the fundamental importance of the discrimination between a tropism and *Unterschiedsempfindlichkeit* is at once obvious. Since the excised muscle is above suspicion of possessing a human soul it might be inferred that for the understanding of the corresponding type of reactions of lower animals physico-chemical data might suffice.

<sup>3</sup> Pflüger's Archiv, vol. 66, p. 439.

In a recent paper, "Experiments on the Behavior of Tubicolous Animals," published in this Journal,<sup>4</sup> Hargitt repeats and confirms these simple experiments. He refers to my paper, just quoted, and then starts upon the following discussion:

"There can be no doubt, therefore, that the reaction is not due to simply a difference of light intensity alone. For whether in diffused or direct sunlight whether in natural or artificial light, the response is to the shadow, sudden diminution of light, a purely negative condition. But it may well be doubted whether this can be properly designated as simply negative phototropism or heliotropism" (Hargitt, p. 300).

Whoever designated these reactions of tubicolous worms as negative heliotropism? Should Hargitt really, with my papers before him, state that I had done so? He does not leave his readers in doubt:

"Furthermore it must be recalled in this connection that the particular stimulus involved in these observations, as previously pointed out, is not light at all directly, but the lack of light, or the shadow. Response is, therefore, induced by a negative stimulus, if such an apparent paradox be tolerable in relation to phenomena of behavior. Of course, it is not overlooked that Loeb has designated these and similar reactions as due to 'negative heliotropism.' At the same time it is not clear that in the present case we are dealing with phenomena at all comparable with those associated with negative heliotropisms as ordinarily understood. For, as already observed, the phenomena are not in themselves negative. They are not dependent upon any given degree of light, or rather darkness, but to the suddenness of the change" (Hargitt, p. 316).

As was to be expected Hargitt concludes from this that the theory of tropism is no longer tenable and that we must return to the anthropomorphic viewpoint, for which, as he states, Jennings has already paved the way.

"Under the later development of the theory of tropisms and its extension to the phenomena of animal behavior, its dominance has

<sup>4</sup> Vol. 3, p. 295, 1906.

relegated the earlier views to the limbo of discarded anthropomorphisms so-called. Without essaying any review of the pros and cons of this problem it may be said that already a reaction has taken place and frankness compels a reconsideration of some of these discarded and discredited views. Such a review has already been made by Jennings so far as it relates to the lower organisms, and his conclusion must, it seems to me, be equally true for many if not most higher animals as well" (Hargitt, p. 313).

What Hargitt has done in one case, Jennings has done in a number of cases. He selects reactions of the type  $f\left(\frac{di}{dt}\right)$ , shows that these reactions do not conform with the theory of tropisms but fails to inform his readers that I had pointed out the existence of this type of reaction and their difference from tropisms long before he did. To give an illustration: In a paper on the "Brain Physiology of Worms" I described experiments on Planarians and earthworms, showing that these animals are not or only slightly heliotropic but react to sudden changes in the intensity of light. Such animals become more quiet when the intensity of light is rapidly diminished, become more active when the intensity of light is suddenly increased. The consequence is that places of a relative minimum in the intensity of light act like a trap upon them. To illustrate this effect and the difference of this reaction from that of heliotropic animals I mentioned the following experiment: One half of a glass vessel is covered with black paper, the other half left uncovered. If Planarians or earthworms are put into such a vessel they collect under the covered half. "They come to rest in those regions which are more weakly illuminated than the surrounding areas. The direction of the rays of light is of little consequence."<sup>5</sup> On p. 130 of his recent book on the "Behavior of the Lower Organisms," Jennings describes the same experiment for Stentor and shows that these organisms will go from the light into the dark but not in the reverse direction. "The essential point is the running back into the shaded region

<sup>5</sup> Loeb: *Studies in General Physiology*, vol. i, p. 360. *Pflüger's Archiv*, vol. 56, p. 247, 1894.

without reference to the direction from which the light comes" (Jennings p. 131). Neither in this nor in any other case in which Jennings describes reactions which depend upon sudden changes in the intensity of light (or any other form of energy) does he refer to my previous experiments on this type of reaction.<sup>6</sup>

I think, however, that those who are working in this field should realize that reactions due to rapid changes in the intensity of light or any other form of energy were first recognized as being typically different from the cases of animal tropisms by the author of this latter theory; and that if new cases of *Unterschiedsempfindlichkeit* are found this does not contradict the existence of the reactions of the type of tropisms any more than the existence of the make and break contractions in a muscle contradicts the existence of electrotonic effects in the same organ; or the existence of accelerations contradicts the existence of velocities.

<sup>6</sup> Although Jennings has attacked my views for years he is certainly not familiar with my papers. This is also evident from his erroneous statement of the theory of tropism on p. 94 of his paper, published by the Carnegie Institution in 1904.

## THE MECHANISM OF THE GALVANOTROPIC ORIENTATION IN VOLVOX

BY

FRANK W. BANCROFT

In a recent paper O. P. Terry ('06) has recorded the discovery that "if kept in the dark for two or three days, the [galvanotropic] response of volvox is changed from cathodic to anodic. This may then be reversed at will by exposure to light." He did not, however, attempt to determine the mechanism by means of which this change in the direction of migration of the organism is brought about. As it appeared to me that the best opportunity for studying the nature of galvanic stimulation by the electric current was presented in those cases where a reversal of the galvanotropism was possible, I have investigated the effect of the current upon the flagella of volvox.

The only observation that I know of on the response of the flagella of this plant to the constant current is by Carlgren ('00, p. 57), who was investigating volvox that was typically swimming toward the cathode. He added carmine to the fluid containing the organisms, and says that on several occasions he saw the current produced by the flagella stop at the anode while it continued at the cathode.

### METHOD

The 110 volt power current was led through a water rheostat, milliammeter, pole changer and non-polarizable boot electrodes to the preparation containing the volvox. The colonies were usually examined in a small glass and paraffine trough. When the currents produced by the flagella were to be observed, india ink



was mixed with the fluid in which the plants were swimming. This made the determination of the direction of the effective stroke of the flagella an easy matter. The preparation was covered with a coverglass resting on one or two thicknesses of filter paper, according to whether the organisms were to be held fast by the coverglass, or to be allowed to swim freely. The filter paper was connected with the boot electrodes by means of a hanging drop. The most useful modification of this method was to cut a hole in the center of a circular piece of filter paper about 8 cm. in diameter. This was placed upon a piece of glass, the volvox colonies were put into a hole in the center, covered with a coverglass, and examined upon a revolving microscope stage. The boot electrodes were connected as usual with the filter paper by means of drops. By this means it was not only possible to move the preparation about, and follow the movements of swimming individuals, but the plants could also be turned through any desired angle, and thus the angle which their long axes made with the current lines passing through them could be varied to any extent without changing the current, and while the organisms were kept continuously under observation. The current density varied from about 20 $\delta$  to about 250 $\delta$ , from 40 to 100 $\delta$  being the strength usually employed. The plants were always studied in the water in which they had been living when collected, or in tap water of similar composition.

#### REVERSAL OF GALVANOTROPISM

I have been able to confirm Terry's results concerning the direction of migration in all important respects. Plants that had been kept in the dark or exposed to diffuse daylight all went to the anode, while of those that had been exposed to direct sunlight for half an hour or more, 30 per cent or more went to the cathode. There appeared, however, to be considerable difference between my results and Terry's as regards the intensities of light required for the anodal and cathodal galvanotropism. Thus Terry found that the usual response for volvox exposed to diffuse daylight was cathodic, while sometimes days of exposure to darkness were required to change the response to anodic. On the other hand,

the volvox which I examined normally showed strong anodal galvanotropism after an exposure to even bright diffuse daylight; and exposure to the direct sunlight was required to change the response to cathodal galvanotropism.

#### BEHAVIOR OF THE FLAGELLA

*Volvox*, as is well known, invariably swims with its anterior end in advance. No one has described a backward swimming of the colony as the result of any stimulus. It is accurately oriented by both light (Holmes, '03, p. 320) and the electric current (Carlgren, '00), but no one has worked out the mechanism of either of these orientations. Holmes, however ('03, p. 321), states for the heliotropism that: "We are safe in saying that when *volvox* changes its direction it is because the flagella on the two sides of the organism beat unequally."



Fig. 1 Diagram of volvox, showing the currents in normal locomotion. The feathered arrow indicates the direction of progression of the colony, the other arrows indicate the direction of the currents in the water.

If india ink be added to a preparation containing volvox currents are easily observed beginning at the anterior end and sweeping backward to the posterior extremity on all sides with equal intensity, as indicated by the arrows in Fig. 1. I examined these currents around volvox colonies that were in the act of orienting themselves heliotropically but could make out no differences in the currents on the two sides. I do not doubt, however, that Holmes' statement is correct.

In the case of galvanotropic individuals, however, differences in the currents at the anode and cathode ends of the organisms are easily detected. The most satisfactory way of studying these differences is with volvox mounted in a sufficient thickness of fluid so that it can swim freely. It swims so slowly that its motions

can easily be followed under the compound microscope, without the addition of any sticky substances to the culture fluid.

If now a current be passed through such a preparation of volvox that has been exposed to weak light, strongly anodal colonies can be picked out and investigated. In such colonies it was invariably and frequently observed that the currents stopped or became very weak on the anode side of the colony while it was not changed much at the cathode side. Sometimes there was no change at the cathode side, and sometimes there was a decided increase in the activity of the flagella when the current was made, and during

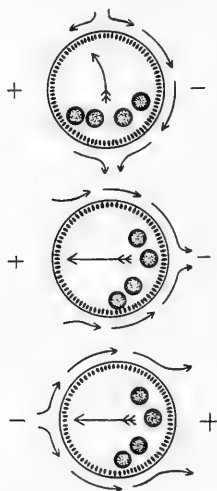


Fig. 2 Diagram of volvox, showing the direction of the currents in the surrounding medium when the colony is swimming in a constant current. 1, anode on the side. The currents stop on that side and the colony turns toward the anode. 2, anode at anterior end. There are no currents at the anterior end, and the colony swims toward the anode. 3, the current has just been reversed, so that the anterior end is now cathodal. Currents have started at the anterior end, and have stopped at the posterior (now anodal) end.

the flow of the current. The same change in the behavior of the flagella was observed at the two poles no matter what the position of the organism. If it is swimming at right angles to the current lines then the flagella on the anode side stop, while those on the cathode continue and the organism is rapidly turned toward the anode (Fig. 2), and continues swimming in that direction. When

the plant is swimming toward the anode there is seen to be no current at its anterior end while the current continues normally posteriorly (Fig. 2). If, now, the current be reversed a violent current is initiated at the anterior end and the current at the posterior end stops (Fig. 2). After this reversal the plant usually continues swimming straight forward to the cathode for a short distance, but soon swerves a little to one side or the other and rapidly orients itself to the anode again.

If the colonies have been exposed to sunlight and made cathodally galvanotropic the behavior of the flagella is completely reversed. Exactly the same pictures are presented to the observer but the current now stops on the cathodal side of the organism and the volvox colony becomes oriented toward the cathode. This result is of some importance for it shows that in the case of volvox the direction of migration has not been changed by some quantitative changes in the action of a mechanism which remains fundamentally the same; as has been described by Wallengren ('02, '03) for *Opalina* and *Spirostomum*. In the cases described by Wallengren the forward stroke of the cilia was always produced by the galvanic current at the cathode side of the animal no matter what its orientation; and, as the forward stroke of the cilia is the best criterion we have for stimulation of infusoria (Bancroft, '05), it must be concluded that in all these cases the underlying nature of the galvanic stimulation was the same. In the case of volvox, however, the facts described indicate clearly that in the anodally galvanotropic colonies the pole at which the constant current produces its characteristic effect is the anode, while in the cathodal colonies the same effect is produced by the current at the cathode. In other words the galvanotropism has been reversed by means of a reversal of the pole at which the electric current produces its characteristic effect.

The only other case that I know of in which a reversal of the galvanotropism has been shown to be brought about in this way is that of *Paramecium* in which it was found possible to reverse the galvanotropism by chemical means (Bancroft '06, '06a). In this case it was made probable that the chemical conditions underlying galvanic stimulation were the same no matter whether that

stimulation takes place at anode or cathode and dependent upon a certain definite ratio of the calcium ions to those of sodium, potassium, lithium, ammonium and other metal ions. The current can vary this ratio, as described by Loeb ('05), since many organic ions precipitate calcium. Lack of material prevented an investigation of the effect of chemical substances on the galvanotropism of volvox. But it was found possible to change the pole at which the galvanic current stimulates by means of pressure.

In all of my first experiments, which were made on the *anodal* colonies the coverglass was rested on but one thickness of filter paper so that the colonies were slightly pressed, and were thus prevented from moving. In all of these colonies it was uniformly seen that the *cathodal* flagella stopped contracting during the flow of the current. As the plants swam to the anode it was difficult to see how a stopping of the cathodal cilia could bring about this result, and much time was lost before it was discovered that the pressure just like the bright sunlight had changed the pole at which the current stimulates. What the pressure and the sunlight have in common is hard to see, unless it is that they both produce an intense stimulation and possibly a slight injury.

#### DISCUSSION OF RESULTS

It has been shown that the galvanotropic orientation of volvox is brought about by a cessation or great diminution in the stroke of the flagella at one pole of the organism. This diminution in activity of the flagella appears to be the only way in which volvox is capable of responding to stimuli. Nothing in the nature of a motor reflex has ever been observed in this organism so far as I know. The flagella always strike most strongly backward. We have then the simplest possible kind of a mechanism for bringing about galvanotropic orientation. The current diminishes the activity of the flagella at one pole of the colony and consequently the activity of the flagella at the other pole cause the organism to turn in that direction. We have here a tropism reduced to its lowest terms. There is nothing of the nature of trial and error present at all.

The fact that nothing of the nature of a motor reflex or reversal in the direction of the effective stroke of the flagella has been observed makes it very probable that, as indicated by Holmes, the heliotropic orientation is also brought about by differences in the strength of the stroke on the sides toward and away from the light. It would seem then that in the case of the orientation of *volvox* to light we have also a tropism pure and simple without any indication of orientation by trial and error.

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# THE INFLUENCE OF EXTERNAL FACTORS, CHEMICAL AND PHYSICAL, ON THE DEVELOPMENT OF FUNDULUS HETEROCLITUS

BY

CHARLES R. STOCKARD

WITH SEVENTEEN FIGURES

The following experiments have been undertaken to determine to what extent the form of the embryo and its manner of development might be modified by external influences. In a previous paper ('06), I have shown that lithium chlorid produces a definite effect on the development of *Fundulus heteroclitus*, as Herbst ('92) had shown for the sea-urchin and Morgan ('03) for the frog. During the past summer I have been able to show that these abnormalities are not only definite but specific for the lithium ion in its action on this egg.

It has also become desirable, owing to recent work on the subject, to determine the permeability of the membrane of *Fundulus* eggs to the various salts; as well as to study the separate and combined effects of osmotic pressure and chemical actions on the development. The eggs of *Fundulus*, as has often been recorded, develop almost equally as well in sea-water, concentrated sea-water, fresh or distilled water, and even, as Morgan ('06) has recently mentioned, out of water.<sup>1</sup> Thus they furnish excellent material for a study of the actions of both hypertonic and hypotonic solutions.

The experiments were performed at Wood's Hole, Mass., while occupying a table kindly furnished me by the Vassar Brothers'

<sup>1</sup> In regard to the development of these eggs in fresh water, Loeb ('94) states: "In fresh water the embryos hatch just as rapidly as in normal sea-water. The fish is able to live in fresh water." On the contrary, I have shown (Stockard, '06), that these eggs are always slower to hatch in fresh water and further that the newly hatched young soon die when left in this medium. Sumner ('06) has also shown that the adult fish is unable to survive in perfectly fresh water.



Institute of Poughkeepsie, N. Y. I wish to express my thanks to this Institution, as well as to the authorities of the Marine Biological Laboratory, for the working facilities furnished me while there. I am also glad to express my indebtedness to Prof. T. H. Morgan for many helpful suggestions, and to Prof. A. P. Mathews for assisting me in calculating the osmotic pressures of my solutions.

#### METHOD AND MATERIAL

As stated above the eggs of *Fundulus heteroclitus* lend themselves peculiarly to such investigations as this. They are hardy and develop in different strengths of almost any solution applied. The action of a salt in sea-water solutions is generally more or less modified, owing to the presence of the salt constituents of the sea-water itself, but this effect can be controlled by the use of the distilled water solutions.

The spawning season of this fish begins at Wood's Hole about the middle of June and extends well into the first part of August, thus giving an opportunity to repeat the experiments many times and to test any uncertain points which might arise. In fertilizing the eggs I have found it advantageous to strip them from the female directly into a dry bowl and then apply the milt from the male, stirring the eggs well so as to mix them thoroughly with the milt. They are left to stand for five or ten minutes when water is added. This method insures a larger percentage of fertilized eggs than will result if they are under water when the spermatozoa are applied. In those cases where the eggs were to be treated with solutions of salts in distilled water they were placed, after being fertilized "dry" directly in fresh water, thus little if any salts could have reached the eggs after they had been removed from the body of the fish.

The time elapsing between fertilization and hatching varies considerably with the temperature, season, etc., being from about eleven to eighteen days.

The salts used in these experiments were with few exceptions fresh Kahlbaum preparations. High percentage solutions were prepared and these were diluted to the proper strengths from time

to time as the experiments required. In discussing the experiments the percentage solutions are expressed in gram-molecular terms. The sugar solutions were prepared fresh for each experiment since they soon became acid with a fungus-like growth if kept for any length of time. Cane sugar inverts to some extent in solutions and may thus vitiate the calculations for osmotic pressures.

It was found that the amount of solution and the number of eggs in the bowl affected to a greater or less degree the rate of development. A large number of eggs in a bowl almost full of liquid develop more slowly than fewer eggs in less liquid; this is due to a difference in oxygen supply as will be shown below. In the same experiment, therefore, approximately equal numbers of eggs and equal amounts of liquid were placed in each bowl. Since there is some individual variation in the eggs from different females the experiment and control were as far as possible from the same batch of eggs.

#### PERMEABILITY OF THE EGG MEMBRANES

A recent paper by Brown ('05), questioning the permeability of the egg membrane of *Fundulus* necessitates a discussion of this subject. The solution of this question is also essential in order to properly interpret my experiments.

Loeb in 1893 showed that diffusion through the egg membrane of older embryos occurred very readily. The addition of 3 grams of KCl to 100 cc. of sea-water brought the heart of a *Fundulus heteroclitus* embryo to a standstill in a few minutes. A considerable amount of the salt must, therefore, diffuse through the egg membrane in a very short time. I repeated this experiment in the following manner: Seven five-day embryos were placed in a 0.67 m (about 5 per cent) solution of KCl. Within ten minutes the heart action of one had ceased, another stopped in eleven minutes, and three others in twelve minutes. The heart's action becomes first periodic and jerky and then gradually stops, though it will often continue to give weak irregular contractions at intervals of one or two minutes for some time after it has apparently stopped. The embryos began to wriggle after having been in the solution only three or four minutes. When the hearts of all the individuals

had stopped beating, within fifteen minutes, the eggs were returned to sea-water; thirty-five minutes later one heart was contracting almost normally, while another was beginning feebly, the others had not recovered even after an hour. On examining the seven embryos the following morning all had entirely recovered. The result demonstrates the readiness with which salts permeate the membrane in eggs a few days old.

In 1903 Brown recorded the results of experiments, showing the immunity of *Fundulus* eggs and embryos to electrical stimulation. For these experiments he used eggs at various stages but some were tried when in the two-cell stage. These experiments, as Brown concluded, go to show the permeability of the membrane during the first hours of development. He states that the most probable explanation of the immunity of these eggs to electric currents as well as to osmotic changes of the medium in which they live is that the membranes of the egg are so freely permeable to ions and possibly to neutral particles that no polarization can occur. "There is a gradual increase in susceptibility to osmotic changes and to the electric current as the embryo develops, the adult being readily stimulated by the current from a single cell, which is quite without action in the embryo."

With the above results and interpretations in view, Brown ('05) has since, from far less convincing experiments, arrived at opposite conclusions. He claims now that the membrane of *Fundulus* eggs is practically impermeable to salts and water during the first six or eight hours of development; since eggs placed in distilled water do not lose their salts during that period. It would be surprising if these eggs did lose their salts in distilled water as they are capable of normal development in this medium. Very probably the inorganic salts of this egg are held in combination in the protoplasm so that they are not able to diffuse out in hypotonic solutions and the readiness with which the membrane is penetrated makes the osmotic pressure low. The fact that the conductivity of the distilled water containing the eggs increased after the first eight hours is probably due to an excretion from the eggs. They undoubtedly give off some waste products as an odor is often observed when a bowl containing eggs is uncovered after standing overnight.

The extensive treatment of *Fundulus* eggs with salt solutions which Mathews ('04)<sup>2</sup> has recorded goes to show that the egg membrane is easily permeable.

To demonstrate further the permeability of the membrane, during the first hours of development, I carried out the following experiment. Since embryos had been found to be affected in a definite manner by solutions of LiCl below the strength of  $\frac{1}{4}$  m, I determined to subject them for short periods while in the two-cell stage to strong solutions of LiCl. Eggs were placed in a molecular and a double molecular solution of this salt in distilled water; one hour and ten minutes later they were all quite abnormal, showing the lithium effect. Some of these were then transferred to sea-water and on examination, eleven hours later, still showed the lithium abnormalities. Those left in the LiCl solution were all badly plasmolized or shrunken while the blastodermic cap was heaped up upon the top of the yolk, almost pinching away from it. Some eggs were removed from the double molecular solution after staying two and one-half hours in it, these failed to recover, and were in the same condition after sixteen hours as those still in the solution. Those removed from the molecular solution after one hour were in the following condition after forty hours; many were dead but some had recovered and showed the embryonic thickening forming on the egg. Of those that spent two and one-half hours in the solution one or two were still living though abnormal, the germ ring having descended only one half of the way down the yolk, and in one case an embryonic shield had formed.

Those taken from the double molecular solution after one hour were, forty hours later, almost all dead, the few living ones being very abnormal. Those that remained two and one-half hours in this solution were all dead.

<sup>2</sup> Mathews tried with these eggs to ascertain the relation if any between the properties of the elements and their physiological action. He concluded that the poisonous action of any cation or metal upon the eggs varied inversely with the solution tension. "Those ions with a very low solution tension are very poisonous; those with a high tension are relatively inert. The poisonous action of any anion also follows this rule." Further "there is an inverse relationship between atomic volume and poisonous action; and a direct relationship between equivalent weights and poisonous action. Poisonous action of the metals is a periodic function of their atomic weights. Elements which have a low atomic volume and high equivalent weight, as mercury, are more active than those with a high atomic volume and a low equivalent weight, as sodium." Many exceptions to the foregoing were found.

After seventy hours those that spent one hour in the molecular solution produced some living embryos, and though well formed they were slower than the control in their rate of development. Those that remained in this solution for two hours showed only a few living eggs with badly stunted embryos. Of those from the double molecular solution of LiCl, after one hour, only one in twenty was alive, and this one was stunted.

Solutions of double molecular and one and one-half molecular strengths were prepared with sea-water and an experiment similar to the above was conducted with like results, although the eggs recovered somewhat more readily after being removed from these solutions and returned to pure sea-water. This latter fact may indicate that some of the salts of the sea-water tend to counteract in part the effects of the LiCl.

At first sight the above results seem to contradict my former statement to the effect that eggs removed from Li solutions in three, four or five hours showed no toxic effects in their later development. It is recalled, however, that the solutions then used were weak ones, while the above are strong enough to kill all eggs remaining in them.

Such results as these can leave, I think, no doubt as to the fact that the membrane of *Fundulus heteroclitus* eggs is readily permeable to salts during the first few hours of their development. The permeability of this membrane at later stages is also beyond question, and probably it becomes more readily permeable as development advances.

#### THE DEVELOPMENT OF FUNDULUS EGGS OUT OF WATER

The only reference hitherto made to the development of *Fundulus* eggs out of water is that by Morgan ('06), to the effect that these eggs will develop on a glass plate in a moist atmosphere. I undertook to rear embryos in this fashion to ascertain what abnormalities, if any, would result from such treatment. The most interesting result obtained was that although these fish develop to all appearances in a perfectly normal manner, except at a little faster rate, *they are entirely unable to hatch while on the moist plates*. Eggs were kept from June 30 until August 2 in a

healthy condition without the embryos breaking through the membranes. Thus these fish remained enclosed in their egg membrane for thirty-three days while the control had begun to hatch after thirteen days.

As there are some points of interest to be brought out in connection with the details of this experiment it may be briefly described. Eggs were placed, shortly after fertilization, on moist glass with all superfluous water removed, they were arranged so as not to be in contact with one another and then covered with a finger bowl to prevent the evaporation of the surrounding moisture. Other eggs were arranged in a similar manner when in the two-cell stage, while still others at this period were covered by finger bowls which had moist filter paper closely pressed in them, thus insuring a more moist atmosphere about the eggs.

When the eggs were fifty-three hours old the control in sea-water showed embryos distinctly formed with the blastopores closed, those on the moist glass also had their blastopores closed and were slightly in advance of the control in their development. One of the eggs that had become dried and shrunken also showed a normal embryo. At three, four, six and ten days old the ones on the moist glass were continuing to develop normally though at a faster rate than the control. This more rapid development was in all probability due to the better aëration out of water.

When fourteen days old many of the control had hatched. At this time those that were under finger bowls without moist paper had become so dry, although moisture had been added several times, that a number of them died; in one lot fifty-four were dead and only twenty still alive, while another lot had forty-seven living and thirty-four dead. This and other such cases indicate that it is important to keep the eggs moist. The ones supplied with moist filter paper were all alive. None of these eggs out of water had hatched; twenty-three were then taken from the glass plate and put into a finger bowl containing sea-water. Eleven hatched; in five minutes, eighteen were out after ten minutes and the entire lot, twenty-three, were swimming about after being in the sea-water for only eighteen minutes. These fish after hatching seemed further developed than the controls which had also hatched, evi-

dently they had been prevented from hatching owing to their inability to break through the egg membrane when out of water.

On the fifteenth day none on the moist glasses had hatched, with the exception of two which I feel sure came out on account of too much moisture or water having been put about those that were rather dry on the day previous. Thirty eggs were now put into sea-water, three of which hatched after ten minutes, thirteen were out in fifteen minutes, twenty-five in twenty minutes, while twenty-seven of the thirty eggs had hatched within twenty-five minutes. On hatching, the embryos swim directly to the top of the water and to the side of the bowl nearest the brightest light thus showing a negatively geotropic and a positively heliotropic reaction.

At sixteen days old not an egg on the moist glasses had hatched, with the exception of the two which had hatched three days before. Twenty eggs were now placed in sea-water, the first one came out after ten minutes. It seems as a rule to require about ten minutes for the eggs to begin hatching and after this period they come out very rapidly. Fifteen were out after fifteen minutes and all were hatched after being in the water only twenty-seven minutes. On the morning of the seventeenth day two of those on the moist glass had hatched, but again an extra amount of moisture had been put about the eggs on the previous day.

When twenty days old, six days after hatching had begun, all of the controls were hatched, those on the moist glasses were alive and well, though none had hatched. An excess of moisture was added to them while still on the glass and twelve embryos came out within twenty minutes, the moisture was then drawn off and hatching ceased.

When twenty-six days old a few of the eggs began to die, three or four out of fifty died on one glass. This was probably due to want of food as at this time all of the yolk had been absorbed for several days past as seen by comparing Fig. 1 of a newly hatched control embryo twelve days old, with Fig. 2 of a moist glass embryo hatched when eighteen days old (*y*k the yolk mass). A comparison of these two figures will also show how the embryos on the moist plates have continued to grow and develop within the egg membrane. When eighteen days old one of these was equally as large

as an eighteen day control embryo which had been free swimming for four or five days.

When twenty-seven days old, more embryos were dying, but those put into sea-water began to hatch within ten minutes, though they swam abnormally at first, going in a circular or spiral course. This was due to the fact that the fish had become cramped by its twisted position within the egg membrane and for an hour or so after hatching they were unable to straighten themselves, but they finally do so and their movements become normal. When at rest the crooked ones have a tendency to topple over on one side.

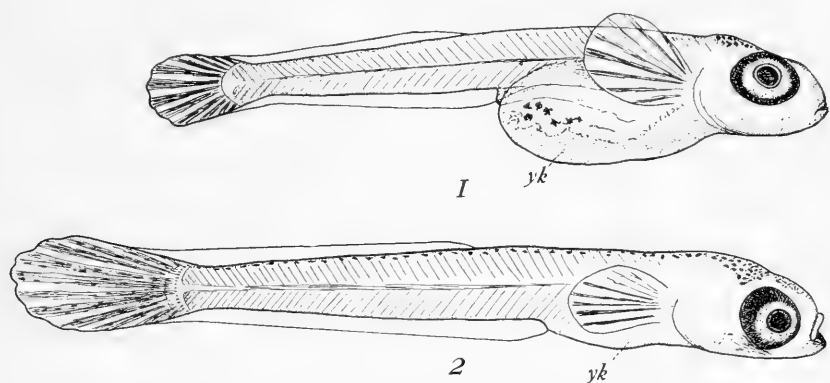


Fig. 1. A normal embryo just hatched, twelve days after fertilization. *yk*, yolk mass.  $\times 17$  diameters.

Fig. 2. A newly hatched embryo which was developed out of water and made to hatch by being placed in sea-water when eighteen days old. *yk*, yolk mass.  $\times 17$  diameters.

After thirty-one days they were still well and unhatched on the glass although a few had starved to death each day. When thirty-two days old, five were placed in sea-water, three came out within twelve minutes and all were out in twenty-three minutes after being put into the water. These had their bodies bent owing to the long cramped position they occupied within the egg membrane, though all soon straightened out and swam normally.



## THE OSMOTIC EFFECTS RESULTING FROM DEVELOPMENT IN SUGAR SOLUTIONS

In order to be able to discriminate between the osmotic effects and the effects of salt solutions it was necessary to make a careful study of the manner in which the eggs reacted to the physical changes; *i.e.*, to the osmotic pressures of the solutions. Loeb ('94) had found that these eggs are "remarkably independent of the concentration of the sea-water." He found that the embryos develop in a perfectly normal fashion in fresh water as well as sea-water to which five grams of NaCl had been added to each 100 cc. of water. When seven and one-half grams of NaCl were added to each 100 cc. of sea-water a blastoderm was still formed, but rarely an embryo. When twenty grams of NaCl were added to 100 cc. of sea-water the power of development of freshly fertilized eggs was annihilated within three or four hours. The only abnormalities that Loeb observed when eggs were in concentrated sea-water were a shrinking of the yolks and a slower rate of development. These experiments fail to show whether the effect is osmotic or chemical, since by using NaCl one is dealing with a chemical poison as well as increasing the osmotic pressure of the solution.

I have used sugar solutions prepared both in fresh and sea-water to determine the effects of osmotic pressure on these eggs. The sugar itself is supposed to exert slight if any chemical action.

The eggs of *Fundulus*, since they develop normally in fresh water, as recorded by Loeb ('02), Mathews ('04), the writer ('06), and others, are evidently insensitive to the lowering of the osmotic pressure of their surrounding medium. This is due probably in part to the inorganic salts of this egg being held firmly in combination with its colloidal organic compounds in the protoplasm.

Seven independent experiments were carried out with sugar solutions in distilled water of the following strengths: 1.53 m, 1.33 m, 1.0 m, 0.66 m, 0.5 m, 0.33 m, and 0.16 $\frac{2}{3}$  m. Solutions of sugar in sea-water were used of the strengths 1.33 m, 1.0 m, 0.88 m, 0.66 m, 0.5 m, 0.33 m, and 0.16 m. A molecular solution of sugar is equivalent to about a 31.4 per cent solution and exerts an

osmotic pressure of 22.4 atmospheres. The osmotic pressure of the sea-water at Wood's Hole, as determined by Garrey ('05) is 21.918 atmospheres, thus being nearly equivalent to a gram molecular solution of sugar.

#### DISTILLED WATER SOLUTIONS OF SUGAR

Several of the sugar solutions employed exert an osmotic pressure lower than that of sea-water, yet owing to their high specific gravity the eggs float on them. We may first consider only the effects induced by the 1.53 m, 1.33 m and the 1.0 m solutions. Two hours after being in the 1.53 m and the 1.33 m solutions the yolks have shrunk in a peculiar way, being circular in outline when viewed from above and oval when seen from the side, having such a form as a plastic sphere would assume if pressed between two horizontal planes. In the molecular solution the yolk does not show this effect to any considerable degree and the rate of development is slightly ahead of the control. After about twenty-three hours all of the eggs in the 1.53 m sugar solution are dead, the membranes of many have burst and allowed the yolk to stream out, while in others the yolk is a small contracted and concentrated mass. At this time many eggs in the 1.33 m are also dead, although the few still alive are further advanced than the control, the blastoderm lies flat on the yolk and the germ-ring is further down. Those twenty-three hours old in the molecular solution are alive and the germ-ring is further over the yolk than in the control. The osmotic pressure of the molecular solution should be about 22 atmospheres which is almost equivalent to the pressure of sea-water, that of the 1.33 m is 26.2 atmospheres, only four atmospheres higher; while the 1.53 m has a pressure of 34.278 atmospheres or about twelve atmospheres above the pressure of sea-water. Eggs in the latter solution are seen to be fatally affected within twenty-four hours. We meet here with the same peculiar problem that I find in looking over Madame Rondeau-Luzeau ('02) and Morgan's ('06) results. They found the upper limit of NaCl on frogs' eggs to be about 2 per cent, which exerts a pressure of 13.61 atmospheres, while Morgan found the upper limit for cane sugar to be 12 per cent, with a pressure of 8.376 atmospheres. I have found

as shown below that *Fundulus* embryos develop in a  $\frac{1}{3}$  m solution of  $MgCl_2$  in sea-water. Here we have a pressure of about thirteen atmospheres above that of sea-water, while as indicated above a pressure of twelve atmospheres more than sea-water is fatal within twenty-four hours when exerted by a cane sugar solution. A possible explanation of such results is that some of the cane sugar becomes inverted and, therefore, exerts a pressure greater than that estimated, since two molecules are now present for each one calculated. The difference in activity which Jenkinson ('06) has lately recorded between isotonic solutions of cane sugar and dextrose on the frog's egg may also be due to the cane sugar having become partially inverted and, therefore, his solutions may not have been isotonic, as he thought; at least this explanation seems just as probable as the one he advances, that the membranes differ in their permeability to the two sugars.<sup>3</sup>

The augmentation of the effect when salts are added to sugar solutions that Morgan ('06) has recorded for the frog and as I shall show below for the fish egg may be largely due to the increased pressure or to the peculiar injurious effect caused by some action of the cane sugar. The sugar used in most of my experiments was crystallized "rock-candy," and was probably pure.

In those solutions of sugar which exert a pressure lower than that of the sea-water the embryos develop normally and often at a rate faster than that of the control. This acceleration probably is due to their floating and hence being better aërated. *Another point of interest regarding eggs in these solutions is that the yolks often become swollen as would be expected in hypotonic solutions.* This observation has never been recorded for these eggs in fresh water nor in any distilled water salt-solutions which were hypotonic to sea water. I have treated them with numbers of such solutions, always making careful study of the structural or form changes which resulted, but in no case have I observed a swelling of the yolk except in *some*, not all, of these hypotonic sugar solutions. This swelling may be due to the sugar becoming inverted

<sup>3</sup> This spring I have had an opportunity to compare the action of cane sugar with simple sugars, such as glucose and levulose, on the frog's egg, and find that the effects of the cane sugar can be explained without assuming any inversion to take place.

after penetrating the membrane. If such should occur, the concentration of the sugar solution within the membrane would be higher than that without and this might produce a strong endosmosis which would result in the swelling of the yolk. The fact that the yolk or egg does not swell in fresh water or in hypotonic solutions has been used as an argument to show that they are immune to osmotic effects. That they are not, however, entirely immune to such effects has been shown above.

#### THE SEA-WATER SOLUTIONS OF SUGAR

The yolks shrink in these solutions in the manner mentioned above, often within one hour after having been put into the solution. The shrinkage of the yolk occurs so promptly that the outer membrane fits loosely around the egg, and often shows an indentation on one side. There occurs below the blastoderm in many cases a bubble-like appearance which disappears, however, as development progresses. When about forty hours old many eggs die in the 1.33 m solution, which exerts a pressure of about twenty-six atmospheres more than ordinary sea-water. The dead eggs usually have a polar ball of protoplasm on the shrunken yolk. The living embryos have the tail end indistinctly indicated and the blastopore remains open much longer than is usually the case. If eggs were removed from these solutions and put into sea-water at any time before twenty hours they soon recovered and developed normally with the exception of those from the stronger solutions, in which case the yolk rarely recovered its full size, and in consequence the pericardium seemed abnormally large. As development continues in the stronger solutions, the yolks become smaller and smaller, and the embryos are likewise much dwarfed with very weak heart contractions which begin only some time after the control embryos have established a free circulation. Fig. 3 shows many of these characters in an embryo when five days old in a molecular solution of sugar in sea-water. Fig. 4 shows the condition of a control embryo of the same age. The body of the embryo is often abnormally bent or twisted on the yolk. The sluggish circulation at times allows the blood to accumulate in certain vessels, commonly in the veins along the ventral line of the tail, as large

red spots. When thirteen or fourteen days old the embryos in the 1.33 m sugar solution resemble the normal embryos of only four or five days. The eggs returned to sea-water after being twenty hours in this solution begin hatching when fourteen days old. Only in the 0.16 $\frac{2}{3}$  m solution of sugar in sea-water were the embryos observed to hatch. The little fish seemed almost normal although they swam peculiarly as their yolks seemed too large and heavy for them to carry.

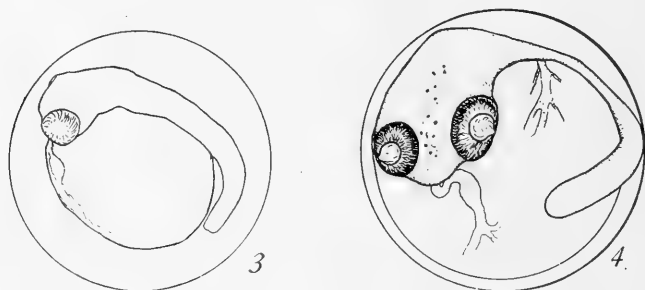


Fig. 3 An embryo from a 0.88 m (30 per cent) sea-water sugar solution five days after fertilization, showing the greatly contracted yolk and the dwarfed condition.  $\times 17\frac{1}{2}$  diameters.

Fig. 4 A normal control embryo of the same age.  $\times 17\frac{1}{2}$  diameters.

The above effects are those expected to result from an increased osmotic pressure, and they go to show that although these eggs are without doubt very resistant to such pressure, it nevertheless exerts an influence on their development. It will be noted by comparing the effects of the sugar solutions in distilled water with those in sea-water that a pressure more than double as strong in sea-water produces a much less injurious effect on the eggs. A not impossible explanation of this peculiar fact is that sugar in the fresh water solutions becomes inverted much more readily than in sea-water.<sup>4</sup> The fresh water solutions of sugar were found to show an acid reaction within a day or two after their preparation, and this acid condition would cause the inversion of the cane sugar. On the

<sup>4</sup> Dr. S. P. Bebee, of the Cornell University Medical College, has analyzed sugar solutions for me with the following results: A 15 per cent distilled water solution of cane sugar becomes partly inverted if allowed to stand for a day or so in the laboratory, while a 15 per cent sea-water solution kept under similar conditions showed no trace of inversion after three days.

other hand sea-water is neutral and the sugar would be much less disposed to invert under such a condition. A fungus-like growth often attacks eggs in fresh water, while I have never observed any such growth about eggs that were immersed in sea-water. The fungus grows in a slightly acid medium and is often present in fresh water solutions of sugar. The acid condition of a solution is in itself injurious. Owing to these conditions a frequent change of the sugar solutions was necessary.

Solutions of glucose and glycerine were also used to test osmotic effects but these chemicals proved to be impure.

#### THE SPECIFIC CHARACTER OF THE LITHIUM EMBRYO

In my paper on the development of *Fundulus* in solutions of LiCl, it was only possible to state at that time that lithium induced certain definite effects which were characteristic of this salt's action. It was also suggested that these effects might be specific for lithium but such could only be known after a number of other lithium salts, as well as a number of the salts of other metals had been used. I have since employed many other metallic salts and have found none of them to produce with any constancy the abnormalities in development which result from the use of lithium salts.<sup>5</sup>

An experiment was first carried out in which eggs were subjected to solutions of  $\text{LiNO}_3$  and  $\text{Li}_2\text{SO}_4$  to ascertain whether the resulting development would be similar to that found in LiCl solutions.

Eggs were placed soon after fertilization in distilled water solutions of  $\text{LiNO}_3$   $\frac{1}{9}$  m and  $\frac{1}{28}$  m, and  $\text{Li}_2\text{SO}_4$   $\frac{1}{18}$  m and  $\frac{1}{50}$  m. The first four or five hours of development was almost normal, but after eight hours those in  $\text{LiNO}_3$   $\frac{1}{28}$  m were beginning to send a projection of the periblast down into the yolk substance, a continuation of this process results in the large bubble-like segmentation cavity before described ('06). When twenty-two hours old the germ-ring is just below the equator in the control (Fig. 9). Those

<sup>5</sup> Madame Rondeau-Luzeau ('02) and Morgan ('06) have found that the upper limit of LiCl that the frog embryo can stand is a 0.65 per cent solution, the osmotic pressure of which is 5.161 atmospheres, while the upper limit of NaCl is about 2 per cent and exerts a pressure of 13.61 atmospheres which is more than double that of the lithium solution. This comparison shows that the effects of the lithium salt are not due to its osmotic pressure alone.

in  $\text{LiNO}_3$   $\frac{1}{9}$  m a few show the blastoderm as a polar ball with large bubble-like segmentation cavity (Fig. 5). Many eggs, however, have polar caps with peripheral germ-rings and a small embryonic shield just beginning to form (Fig. 6). A very few are more nearly normal with the germ-ring extending one-third over the yolk sphere. This is the same condition found in eggs of this age in solutions of  $\text{LiCl}$ , which gives for the first few hours no evident effect, then retards the development, preventing the downgrowth of the germ-ring, and often causing the formation of polar protoplasmic balls with the bubbles beneath, shown in Figs. 1, 2, 3 and 4 in my earlier paper ('06). It might be objected that at this stage of development there are only a limited number of ways that the eggs could be affected. This may be granted, but from what is recorded in other sections of this article it will be seen that there are several possible modifications that may appear, and no other substances have given, with any degree of constancy, the above modifications. Those in the  $\frac{1}{8}$  m  $\text{LiNO}_3$  solutions were in a similar condition.

After twenty-three hours in  $\text{Li}_2\text{SO}_4$   $\frac{1}{8}$  m most of the eggs are dead with polar balls of protoplasm. Of those still alive, most have polar caps with bubbles beneath (Fig. 7). Others have polar caps with peripheral germ rings and embryonic shields just forming (Fig. 8); a very few are more nearly normal and show the germ-ring one-fourth the way down the yolk.  $\text{Li}_2\text{SO}_4$   $\frac{1}{16}$  m has caused similar though less pronounced effects.

The control eggs when forty-six hours old show the embryos distinctly marked out with optic vesicles and lenses visible. In the  $\text{LiNO}_3$   $\frac{1}{9}$  m at this time a few eggs are dead with polar caps; their blastoderms showing a bubble-like appearance beneath. Many have embryonic thickenings forming in the polar caps similar to the condition shown in my former Fig. 18. A few have their caps extending halfway over the yolk with short embryos formed, a few others have the blastopores almost closed although the head end of the embryo is abnormal. In  $\text{LiNO}_3$   $\frac{1}{28}$  m many eggs are dead, a few have their blastopores closed though the head end of the embryo is abnormal with no eyes showing. There are others with the blastoderms only one-half over the yolk and form-

ing short embryos. In  $\text{Li}_2\text{SO}_4$   $\frac{1}{18}$  m some are dead, others have a polar cap with an embryo forming in it. Of those in  $\text{Li}_2\text{SO}_4$   $\frac{1}{50}$  m some are dead, others are with the blastopore almost closed, but the embryos show no optic vesicles as yet, a few have polar caps with embryonic thickenings in them.

When seventy-two hours old, the eggs in  $\text{LiNO}_3$   $\frac{1}{9}$  m have some embryos almost as long as those of the control but with no circula-

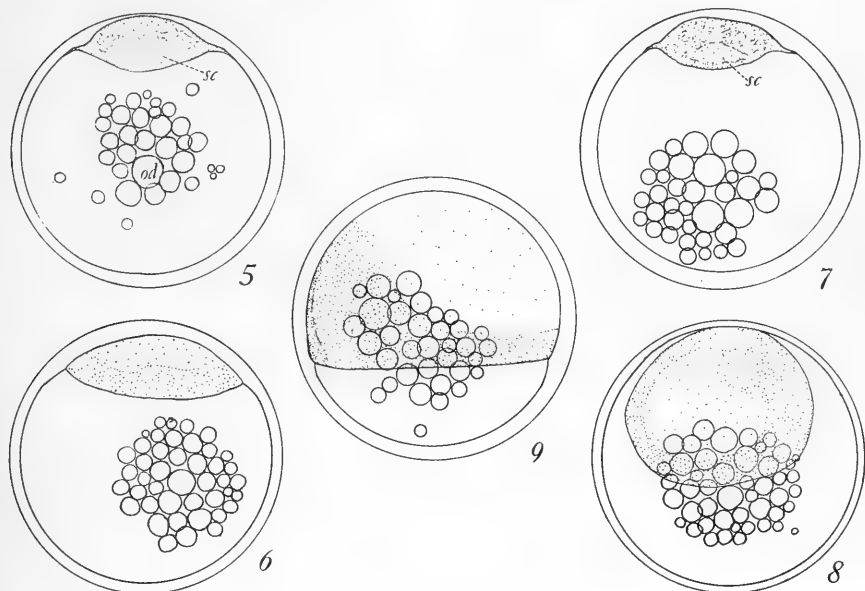


Fig. 5 An egg from a  $\frac{1}{9}$  m  $\text{LiNO}_3$  solution twenty-three hours after fertilization. *sc*, segmentation cavity; *od*, oil drops.

Fig. 6 From a  $\frac{1}{25}$  m  $\text{LiNO}_3$  solution when twenty-three and one-half hours old.

Fig. 7 From a  $\frac{1}{50}$  m  $\text{Li}_2\text{SO}_4$  solution at twenty-three hours old *sc*, segmentation cavity.

Fig. 8 From a  $\frac{1}{18}$  m  $\text{Li}_2\text{SO}_4$  solution at twenty-three and one-half hours old.

Fig. 9 A control egg of twenty-three hours. All magnified  $17\frac{1}{4}$  dia.

tion apparent; the heads are poorly formed and show no optic vesicles in surface view. In the  $\text{LiNO}_2$   $\frac{1}{28}$  m solutions all of the eggs are dead. In  $\text{Li}_2\text{SO}_4$   $\frac{1}{18}$  m some have the germ-rings only one-half way over the yolk, with short embryos formed; in others development has stopped. In the  $\text{Li}_2\text{SO}_4$   $\frac{1}{50}$  m solution most of the eggs are dead though a few have badly twisted embryos with poorly formed heads and no optic vesicles.



At ninety-six hours old, the  $\text{LiNO}_3 \frac{1}{9}$  m embryos show no eyes, the circulation of the blood can not be detected, while in the control it is very distinct. The pigment spots are scarce. The  $\text{Li}_2\text{SO}_4 \frac{1}{18}$  m eggs have short embryos with their blastopores still open.

When eight days old, those eggs in  $\text{LiNO}_3 \frac{1}{9}$  m have short embryos with poorly formed eyes, they are pale in appearance, the heart beats slow the blood is colorless, and the tail unusually bent. Comparing this general description with the detailed one recorded for the development of this fish in solutions of  $\text{LiCl}$  it will be found that the development of the egg of *Fundulus* is as characteristic in solutions of lithium salts as is that of the frog under like conditions as recorded by Gurwitsch ('95, '96), Morgan ('03, '06) and others.

#### THE EFFECTS OF METALLIC CHLORIDS ON THE DEVELOPMENT OF *FUNDULUS* EGGS

A number of chemical solutions have been employed singly and mixed in order to further analyze osmotic and chemical action, as well as to distinguish if possible any definite morphological response that might result from the action of any one salt. The notes on these experiments have become so voluminous that it is inadvisable to attempt to record them all. I shall, therefore, state as concisely as possible the factors involved and the chief results that followed.

Loeb's ('93) experiments with  $\text{KCl}$  were repeated:<sup>6</sup> the concentrations of the solutions used being  $\frac{1}{2}$  m,  $\frac{3}{8}$  m and  $\frac{5}{8}$  m in distilled water and  $\frac{1}{2}$  m,  $\frac{3}{4}$  m, and molecular in sea-water. Many of the eggs in the stronger solutions died during their early development.

<sup>6</sup> Loeb observed the interesting fact that *Fundulus* embryos would develop in solutions of  $\text{KCl}$  without circulation of the blood taking place. The heart was entirely still and the blood failed in consequence to move through the vessels. He stated that in these cases the blood system developed normally, the only peculiar point being that the pigment spots did not migrate to the blood vessels and arrange themselves along them as they usually do. I find that the circulatory system does develop to some extent but by no means normally as may be seen by a casual examination of the heart. Hence possibly the failure of the pigment cells to migrate. Loeb also found that embryos four to six days old were killed by remaining one hour in a 1.5 per cent solution of  $\text{KCl}$  as a result of the effect of this salt upon their heart's action, while if put into a 5 per cent solution after fertilization they live and develop.

Solutions of KCl in distilled or sea-water caused the eggs to develop at a slow rate, the yolks were shrunken and bubble-like appearances were often seen below the embryonic shield. When studied in section these eggs showed the following conditions: The periblast beneath the embryonic shield had become vacuolated with huge cavities in it as shown in Fig. 10. It thus bulged into the yolk mass and the cavities produce the bubble-like appearance

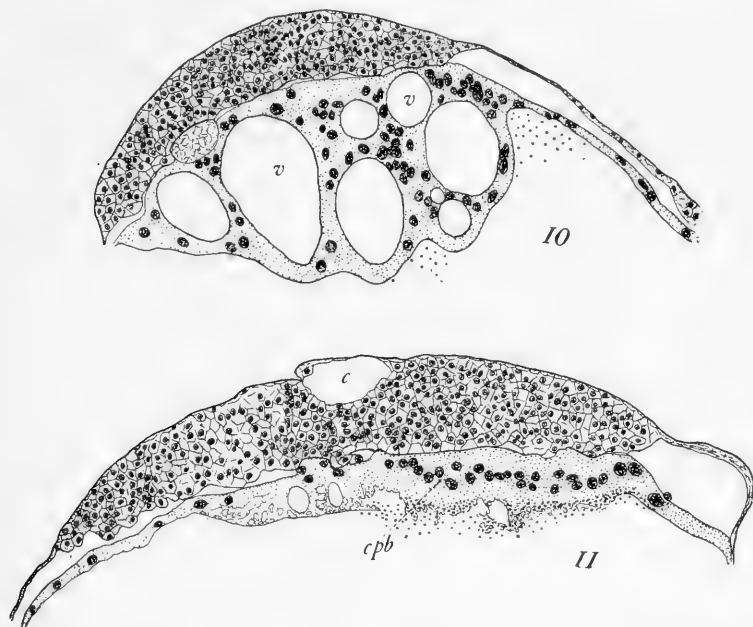


Fig. 10 A section of the embryonic shield from an egg thirty hours old in a  $\frac{3}{4}$  m sea-water solution of KCl, showing huge vacuoles *v* in the periblast beneath the shield.  $\times 58\frac{1}{3}$  diameters.

Fig. 11 A section of the blastoderm of an egg thirty-one hours old that had spent the first twenty-three hours after fertilization in a  $\frac{1}{2}$  m  $\text{MnCl}_2$  solution. The central periblast, *cpb* shows much thickened, with many large nuclei accumulated in this region. *c*, Cavity in the blastoderm.  $\times 58\frac{1}{3}$  diameters.

seen in living eggs. The embryos are always much dwarfed and pale. The heart never contracts although the embryo may remain alive for as long a period as two weeks. The pericardium is often puffed out and is unusually prominent, as also occurs in some other solutions, as shown in Fig. 12, *pc* for an embryo from a mixture of  $\text{MgCl}_2$  and  $\text{NaCl}$ .

Eggs that remained as long as thirty hours in  $\frac{1}{2}$  m distilled water solutions of KCl would recover if placed in sea-water. Other eggs were left for three days in KCl  $\frac{3}{8}$  m distilled water solutions, and afterward recovered, the heart beginning to beat, etc., when returned to sea-water. Normal embryos several days old were very readily killed if subjected to even weak solutions of KCl; their heart's action being stopped. It thus seems as though an embryo may live and develop without its heart ever having contracted, but if it had once begun to contract any cause that may stop this contraction proves fatal. My results then in a general way agree with Loeb's observations though I should take exception to his statement that the circulatory system develops normally even though the blood does not circulate.

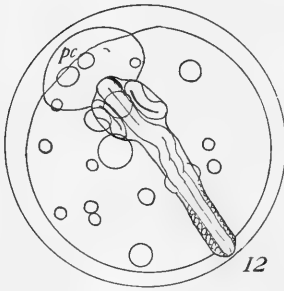


Fig. 12 An embryo from a mixed solution of  $\text{MgCl}_2$   $\frac{1}{3}$  m +  $\text{NaCl}$   $\frac{1}{3}$  m when forty-three hours old, showing swollen, pc, pericardium.

The major parts of the system do seem to develop but by no means normally, the heart being small and weak and it is often only a straight tube with the balloon-like pericardium surrounding it. Many clots of red corpuscles are noted in several of the sinuses. The above facts are also of interest in connection with Howell's analysis of the inhibitory action of the vagus nerve on the heart beat as being due to the liberation of K-ions about the nerve endings.

A mixture of a  $\frac{1}{2}$  m KCl and a molecular NaCl solution was prepared with 60 cc. of the former to 10 cc. of the latter. This mixture showed the same general effect on the eggs as the simple KCl solution; the blastoderm bulged up slightly and the yolks were shrunk. Many embryos, however, seemed stronger and better developed with more pigmentation and with larger red blood clots. Some of these embryos were placed in sea-water when seven days old but failed to recover.

A  $\text{CaCl}_2$   $\frac{1}{3}$  m solution in distilled water proved highly toxic. The blastoderms flattened down, the cells apparently spreading unusually far apart. The eggs died within about twenty-four

hours. Eggs that were subjected to  $\frac{1}{8}$  m  $\text{CaCl}_2$  one hour after fertilization were almost all dead within four hours, the living ones were abnormal, and all died after twenty-four hours. This result further illustrates the readiness with which the egg membrane is penetrated during the first few hours after fertilization. A mixture of 60 cc.  $\frac{1}{8}$  m  $\text{CaCl}_2$  and 10 cc. 1.0 m  $\text{NaCl}$  proved equally as fatal as the  $\text{CaCl}_2$  alone had done, neither of the cations seem to exert an anti-toxic action toward the other.

Solutions of  $\text{NH}_4\text{Cl}$  in distilled water of concentrations  $\frac{1}{10}$  m,  $\frac{1}{7}$  m,  $\frac{1}{5}$  m,  $\frac{1}{4}$  m,  $\frac{1}{3}$  m and  $\frac{1}{2}$  m were used; a molecular solution of  $\text{NH}_4\text{Cl}$  is equivalent to about a 5.05 per cent solution. Sea-water solutions of  $\frac{1}{3}$  m,  $\frac{1}{2}$  m and  $\frac{3}{4}$  m were also employed. These solutions seemed to cause the yolk to shrink slightly, the blastoderm to thicken so that on examining the living eggs one would think that the segmentation cavity was abnormally large as it is in the lithium embryos. On studying sections of these, it was found that the cells are loosely connected, making the blastoderm unusually thick so that it projects down into the yolk. The segmentation cavity is, therefore, not abnormally large as in the lithium embryos. Many of the eggs die at various stages. The rate of development is retarded and the blastopore is slow to close. Many of the embryos are short with their tail ending abruptly. In some embryos the heart beats slowly and the circulation is sluggish; in others there is no pulsation at all, and still others in the same solution may show a very good circulation. Embryos lived as long as eighteen days in such solutions but failed to hatch.

Such short embryos as those above described seem to result from any cause that retards development and prevents the normal down-growth of the germ-ring. When such embryos were removed from  $\frac{1}{2}$  m  $\text{NH}_4\text{Cl}$  solutions when forty-three hours old and placed in sea-water, they recovered in one day and hatched when fourteen days old. The embryos in  $\text{NH}_4\text{Cl}$  are always dwarfed, with poor circulation, lightly colored blood, and sparse pigmentation, having a pale appearance. The sea-water solutions of  $\text{NH}_4\text{Cl}$  were much less toxic than the distilled water ones.

Mixtures of  $\text{NH}_4\text{Cl}$   $\frac{1}{5}$  m +  $\text{MnCl}_2$   $\frac{1}{20}$  m,  $\text{NH}_4\text{Cl}$   $\frac{1}{4}$  m +  $\text{MnCl}_2$   $\frac{1}{25}$  m and  $\text{NH}_4\text{Cl}$   $\frac{1}{7}$  m +  $\text{MnCl}_2$   $\frac{1}{20}$  m were tried. The

eggs lived better in these solutions than in either the  $\text{NH}_4\text{Cl}$  or the  $\text{MnCl}_2$ . The first twenty-four hours of development is almost normal though some eggs die, the rate of development after this period becomes retarded, the embryos have swollen pericardia in some cases, and are pale and small. Some of them continued to live in these solutions for fifteen days but were far from the hatching stage at this time. The weaker toxicity of these mixtures when compared with the action of the salts used singly may be due as Loeb ('02) has claimed, to an anti-toxic effect of one ion on the other. The mixtures with less  $\text{NH}_4\text{Cl}$  were always less active. Loeb found the bivalent cations to show an anti-toxic action toward the monovalent ones.

$\text{MnCl}_2$  solutions were used of the strengths  $\frac{1}{25}$  m,  $\frac{1}{16}$  m, and  $\frac{1}{12}$  m in distilled water; and  $\frac{1}{16}$  m,  $\frac{1}{12}$  m, and  $\frac{1}{8}$  m in sea-water. Eggs that were subjected to the action of such solutions responded in the following way: Those in the distilled water solutions go normally for several hours, then when about eighteen or twenty hours old the blastoderm shows a dark central portion when viewed from above, while in side view it shows that the dark area protrudes downward into the yolk. A section of such a blastoderm is seen in Fig. 11. The dark area is shown to result from an unusual thickening in the center of the central periblast, *cpb*, and the accumulation at this point of a number of the large periblast nuclei. A slight cavity, *c*, is not uncommon near the surface of the blastoderm. This unusual thickening of the periblast seems to render difficult the subsequent descent of the germ-ring, and development is thus slightly retarded. When about forty hours old many embryos have their germ-rings only one-half over the yolks, and a short embryo is outlined on the embryonic shield. Many of the eggs died in these solutions. The embryos have a feeble pulse and the blood is often clotted in some of the larger vessels. When fifteen days old embryos hatched in the  $\frac{1}{25}$  m solutions but swam abnormally; one embryo was seen to hatch in a  $\frac{1}{16}$  m  $\text{MnCl}_2$  solution but it was entirely unable to swim.

The solutions of  $\text{MnCl}_2$  in sea-water formed slight precipitates and the results are thus no doubt vitiated to some extent, nevertheless the dark central portion of the blastoderm always showed.

Short embryos with open blastopores were formed in many cases. The heart was weak and tubular with feeble contractions and was surrounded by a swollen pericardium. One embryo when eleven and one-half days old hatched in a  $\frac{1}{5}$  m solution, but was unable to swim. In many of these embryos no heart beat could be detected and the yolks were badly shrunken. In one case a one-eyed embryo was noted, this is mentioned on account of the tendency of magnesium salts to produce such a condition, but the eye structures of this embryo were very imperfect and no lens was present, this condition will be found to differ entirely from that described below as caused by the action of  $\text{MgCl}_2$ .

Eggs were subjected to  $\text{MgCl}_2$  solutions of the following strengths  $\frac{1}{10}$  m,  $\frac{1}{5}$  m,  $\frac{1}{3}$  m, and  $\frac{1}{4}$  m in distilled water, and 0.238 m, 0.25 m, 0.286 m, 0.33 m and 0.5 m in sea-water; a molecular solution of  $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$  being equivalent to about a 20.3 per cent solution. The early development in all of these solutions is strikingly normal considering the large death rate which occurs during these stages. The salt seems especially toxic to the early embryo. At seventy-four hours some embryos are well formed, though behind the control in their development, and the blood circulation is slow in some while others have a quick heart action. When ten days old all are weak and smaller than the control, the blood flow is slow and spasmodic; in some embryos the circulation has ceased and the blood is collected in the sinus and heart and appears as a red streak in front of the head. Many of the livelier embryos wave their pectoral fins.

In the  $\frac{1}{5}$  m and  $\frac{1}{3}$  m distilled water solutions many embryos hatch when about fifteen days old, though they swim abnormally on account of their bodies being twisted. The sea-water solutions cause the yolks to shrink and in these the embryos are also small with sluggish circulations. Although kept alive for twenty-four days none of the eggs in the sea-water solutions would hatch.

The conditions cited above are general and occurred also in a number of different salt solutions, but the condition which may now be considered seems peculiarly characteristic of the Mg salt. In the  $\frac{1}{5}$  m sea-water solutions one-eyed embryos occurred with surprising regularity in 50 per cent of the eggs. This experiment

was repeated three times and each time it so happened that exactly one-half of the embryos had only one eye. These cyclopean fish were rather abnormally shaped though they were able to twist about and wave their pectoral fins vigorously. The other embryos were apparently normal in all particulars, the magnesium seeming not to have affected them.

In sections the one-eyed condition was found to result from the union or fusion of the Anlagen of the two optic vesicles. Cases were found illustrating various degrees in this fusion, it seemed as though the optic vesicles were formed too far forward and ventral and thus their antero-ventro-median surfaces fused. This condition results in one large optic vesicle which in all cases gives more or less evidence of its fused or double nature.

As a rule but a single lens is formed, the size of which depends upon the size of the optic cup or more exactly upon the size of the ectodermal area influenced by the optic cup to form a lens. This lens formation is interesting in connection with the results of the experimental work of Lewis ('04) and others on the lens development in Amphibians. I ('07) have entered into a more detailed discussion of this subject elsewhere.

The lens was found to show a double or fused structure in one case out of the ten embryos that were sectioned; the other portions of this eye were also more distinctly double than was usually the case. This condition represents the last step in the fusion of the two eyes, slightly greater fusion would result in a single eye.

With no other solution has such a condition as the above been procured, and its abundant occurrence in sea-water solutions of  $\text{MgCl}_2$  strongly indicates that this one-eyed condition is characteristic of the action of such solutions on the developing *Fundulus* embryo.

Solutions of  $\text{MgCl}_2$   $\frac{1}{10}$  m +  $\text{NaCl}$   $\frac{1}{5}$  m in distilled water, and  $\text{MgCl}_2$   $\frac{1}{5}$  m +  $\text{NaCl}$   $\frac{1}{5}$  m in sea-water were tried on the eggs with the results following:

The distilled water mixture produced no effect on the development, nor do such strengths of the two salts employed separately.

The sea-water mixture contained twice as much  $\text{MgCl}_2$  as the distilled water one. The results are instructive. When eighteen

hours old the blastoderms were raised up prominently on the yolks. Many eggs died during early cleavage, and altogether the eggs are decidedly abnormal. Neither of these salts acting alone would give such an effect. When forty-two hours old the yolks are shrunk and all of the embryos have a balloon-like pericardium in front of the head, Fig. 12, *pc*. Later, the circulation often becomes feeble. This occurs also in simple  $\text{MgCl}_2$  solutions.

When nine days old the embryos are small and the yolks shrunk. All steps of the fusion of the two eyes into one are shown. This condition makes it certain that the magnesium of the mixture has acted upon the embryos. After fifteen days the eggs are still alive, though small and pale. Thus this double solution is more active than a simple  $\text{MgCl}_2$  solution and produces magnesium effects with really less magnesium present than is necessary to give a like result when  $\text{MgCl}_2$  acts alone in sea-water. It was stated above that a strength of  $\frac{1}{4}$  m  $\text{MgCl}_2$  in sea-water was the weakest solution that caused the one-eyed embryo. The fact that in the mixture a  $\frac{1}{5}$  m  $\text{MgCl}_2$  sea-water solution gives a like effect may be due to the additional osmotic pressure exerted by the  $\text{NaCl}$  present as has been suggested by Morgan ('06), to explain similar phenomena in the action of salt solutions on frog eggs. It may also be suggested that the  $\text{Mg}$  ions act against the  $\text{Ca}$  ions of the sea-water and thus permit the  $\text{Na}$  ions to become more active, but this explanation will certainly not apply here, since the embryos show characteristic magnesium effects.

Eggs were subjected to distilled water solutions of  $\text{NaCl}$   $\frac{1}{5}$  m,  $\frac{1}{4}$  m and  $\frac{3}{8}$  m. During the first day of development many died in most of these solutions. When the eggs were forty-eight hours old the  $\frac{3}{8}$  m solution contained many dead eggs, although the few still alive were almost normal in appearance. This solution contains only 2.19 per cent  $\text{NaCl}$  which is less than the amount in normal sea-water yet it is obviously toxic to these eggs. It is evident that other salts present in the sea-water counteract this toxic effect of  $\text{NaCl}$ . When fourteen days old all of the living embryos appear normal. The  $\frac{3}{8}$  m solution contained one hatched embryo which had a slow pulse and feeble fin movements, it lay at rest on one side but moved if pricked with a needle. In the  $\frac{1}{4}$  m solution of  $\text{NaCl}$



more embryos had hatched than in the control though all of these fish swim with a jerky motion often moving in a spiral course or even turning somersaults in the water. The salt seems to act either upon the nerves or muscle fibers of the embryo causing the nervous twitching or jumping movements. The pectoral fins seem to lack their usual coördination. This condition is not induced by the absence of some constituent of the sea-water since embryos hatched in distilled water swim normally. The result is then undoubtedly due to the action of the NaCl. The embryos die within one or two days after hatching with their bodies peculiarly curled or twisted. Jenkinson ('06) has lately recorded a similar twisting and inability to swim for newly hatched tadpoles in NaCl solutions.

Sea-water solutions of  $\frac{2}{3}$  m,  $\frac{5}{8}$  m and molecular concentrations of NaCl showed only a tendency to shrink the yolk. The development progressed almost normally and only a few eggs died. On their shrunken yolks the embryos when six days old were small and behind the control in their development. At fourteen days the embryos hatched in the  $\frac{2}{3}$  m and  $\frac{5}{8}$  m solutions, those in the weaker solution swam normally while those in the stronger showed the same jerky motions described above. On comparing these effects with those in the distilled water solutions it is reasonable to suppose that some constituent of the sea-water is capable of counteracting the effect of NaCl up to a given point<sup>7</sup> but when an excessive amount of the salt is present its action is not entirely checked.

Eggs lived for twenty-four days in a healthy condition in the molecular NaCl solution although none of them hatched. Loeb kept eggs as long as five weeks in a NaCl solution in sea-water without hatching.

Embryos three days old were subjected to a double molecular

<sup>7</sup> In 1902 Loeb found that *Fundulus* embryos would not develop in a solution of NaCl in distilled water equivalent to the concentration of NaCl in the sea; he then added a trace of calcium salt and found development to be normal. After a number of experiments the conclusion was reached that the salts of monovalent cations with monovalent anions exert a toxic effect at certain concentrations. This toxic effect could be annihilated through the addition of a small amount of a salt having a bivalent cation or by a still smaller amount of one having a trivalent cation. In other words, the antitoxic effects of cations vary directly as the valence of the elements. It was also found that mono-, bi-, or trivalent anions were all unable to produce a like effect.

solution of NaCl in sea-water and they continued to develop in an apparently normal fashion but with their yolks shrunk. Loeb ('94) had found that embryos three or four days old might be placed into a 27½ per cent sea-water solution of NaCl and continue normal development. None of these embryos, however, will hatch.

In several of the NaCl solutions I found embryos that lacked all skin pigmentation thus appearing almost white, these were not true albinos, however, since their eyes showed pigment. Such pale embryos hatched when returned to sea-water.

After a consideration of the foregoing results one must admit, it seems to me, as probable that some of the elements exert a specific stimulus on the fish embryo and cause it to develop in a characteristic manner. LiCl, KCl, MnCl<sub>2</sub> and MgCl<sub>2</sub> seem to induce rather constant and definite effects or types of embryos. The form of the embryo seems to be influenced by external factors in development as well as by internal ones; in other words, the chemical environment of an egg is important in determining the final resultant of the factors in inheritance.

It may be suggested as a probability that every element that forms a chemical union with the germ substance produces on the developing egg through its action definite anatomical and physiological effects, which of course will vary in different kinds of eggs. Thus since the normal form of an animal may be altered in a definite way by certain chemical actions of the elements, we may assume that the specific nature of any animal is a product of the chemical composition of the egg cell from which it sprang.

#### THE ACTION OF MIXTURES OF SALTS IN SOLUTION: THE CHEMICAL VERSUS THE OSMOTIC EFFECTS

The following experiments were conducted in order to determine whether or not by increasing the osmotic pressure of the solution through the addition of a chemically indifferent substance, such as sugar, the chemical action of salts might be augmented. In other words, will eggs become more susceptible to the chemical action of a weak salt solution if the osmotic pressure of this solution be increased? Morgan ('06) has performed similar experiments

with frog's eggs and concludes that in order to be effective the two solutions together must exert a higher pressure than the one producing its effect at the lower limit but less than for the other that produces its effect at a higher pressure. These osmotic pressure effects are somewhat contradictory as I have above pointed out in mentioning Morgan's results in which he finds the upper limit of NaCl to be about 2 per cent with a pressure of 13.61 atmospheres, while a like fatal limit for sugar was found to exert a pressure of only 8.376 atmospheres. It is also recalled that I described above a like contradiction in comparing the pressures of fatal sea-water solutions of sugar with similar solutions of  $\text{MgCl}_2$ . As there stated, this contradiction is possibly due to the fact that the cane sugar in solution becomes inverted and thus the actual pressure is really double that calculated.

In working with *Fundulus* eggs, as has been already pointed out, the experimenter has the advantage of being able to keep them alive in solutions which exert pressures both above and below that to which the eggs are normally accustomed. This fact has been of especial value in analyzing the results of the following experiments. To anticipate what is to follow it may be stated that on adding certain percentages of sugar to a distilled water salt-solution, the action of the salt was increased although the total pressure of the solution was less than the osmotic pressure of ordinary sea-water. Such a result may probably be due to the action which would take place if the sugar became inverted in the solution.

The following distilled water solutions of LiCl + sugar were employed in one experiment, LiCl 0.128 m, 0.096 m, 0.064 m and 0.032 m with 0.44 m of cane sugar in each. All of these solutions exert an osmotic pressure less than that of sea-water, except possibly the first which has an almost equal pressure. After nineteen hours the eggs in LiCl 0.128 m + 0.44 m sugar had polar caps with "bubbles" beneath and many were dead, those in LiCl 0.096 m + 0.44 m sugar were in about the same condition. LiCl 0.064 m + 0.44 m sugar had also produced polar caps and no germ-rings were formed, LiCl 0.032 m + 0.44 m sugar had caused half of the embryos in it to die, while the living ones had formed abnormal germ-rings. Eggs in a solution of LiCl 0.032 m are scarcely

if at all affected at this time, and those in 0.44 m sugar are normal. The eggs continue to show these graded abnormalities in the different solutions and when sixty-eight hours old were as follows: All were dead in the three stronger mixtures, and a few short embryos had been formed and were still alive in the LiCl 0.032 m + 0.44 m sugar. In LiCl 0.128 m at sixty-eight hours many were dead but a good number of short embryos were present; in the LiCl 0.032 m 20 per cent of the embryos were almost normal. In the 0.44 m sugar solution the embryos were normal. The result shows that sugar augments the action of the LiCl although the pressure of the mixed solution is less than that in which the eggs usually live. This conclusion seems to me correct for now I realize the improbability that the sugar may have inverted which would thus have exerted twice the pressure supposed; if this were true then all of the solutions would have a pressure higher than that of the sea-water, though still not high enough in themselves to cause any of the above effects as will be readily seen by comparing the pressures of sea-water solutions in which the eggs develop normally.

A reverse experiment was conducted in which the amount of LiCl present in the solution was constant while varying amounts of sugar were added. LiCl 0.032 m was mixed with 0.293 m, 0.44 m, 0.586 m and 0.88 m sugar, and LiCl 0.016 m with 0.293 m, 0.44 m, 0.586 m, 0.88 m and 1.253 m sugar. The results of these experiments showed as one would expect from the above that the injurious action of the solutions increased with the amount of sugar present, and moreover the activity of the mixture was always stronger than that of either constituent when used alone. The last point is well illustrated by eggs of forty-eight hours in the solution of LiCl 0.032 m + sugar 0.586 m. All the eggs in this solution have the blastoderm in the form of a ball on the upper pole, only a few are still alive and in these the large bubble-like segmentation cavity is present. The osmotic pressure of this mixture is lower than that of sea-water provided that the sugar has not inverted. At this time, forty-eight hours, eggs in 0.586 m sugar solution are all normal, and those in 0.032 m LiCl almost all have their germ-rings three-quarters of the way over the yolks with short embryos formed; some, however, have the germ-ring only one-quarter or one-third of the way down.

Mixed solutions of LiCl and sugar were also prepared in sea-water. A 0.293 m solution of sugar was added to 0.336 m, 0.256 m and 0.192 m solutions of LiCl. The general results agree with those described above for the distilled water solutions, although the contrast between the simple LiCl solutions, and the mixtures was not so sharp. Figs. 13 to 17 of eggs when twenty hours old serve to indicate very well the conditions caused by the solutions at this period. Fig. 13 shows the appearance of the majority of eggs in LiCl 0.256 m + sugar 0.293 m. Fig. 14 shows the egg nearest normal in the same solution. Fig. 15 indicates the stage that the large majority of eggs in LiCl 0.256 without the sugar have reached at this time. A marked difference exists between this embryo and those in the mixture. Fig. 16 is the most abnormal one in the LiCl 0.256 m and Fig. 17 shows a control egg at this age.

Eggs were subjected to the following distilled water mixtures of  $\text{NH}_4\text{Cl}$  and sugar,  $\text{NH}_4\text{Cl}$   $\frac{1}{3}$  m,  $\frac{1}{7}$  m, and  $\frac{1}{10}$  m + 0.44 m sugar. The development of the eggs in these different strength mixtures was as we would expect from the result shown above. Those in the  $\text{NH}_4\text{Cl}$   $\frac{1}{3}$  m + sugar 0.44 m were all dead within nineteen hours with their blastoderms in the form of balls of cells on the upper pole of the egg. At this time some of those in  $\text{NH}_4\text{Cl}$   $\frac{1}{7}$  m + sugar 0.44 m had the germ-ring one-quarter way down the yolk, the majority, however, showed the blastoderms as polar balls which had not flattened down; many were dead. The weakest solution produced fewer abnormalities. The eggs in the 0.44 m solution of sugar were normal at this time, nineteen hours, and those in  $\text{NH}_4\text{Cl}$   $\frac{1}{3}$  m had thirteen normal and seven dead.

When forty-three hours old all of those in  $\text{NH}_4\text{Cl}$   $\frac{1}{7}$  m + sugar 0.44 m were dead, no embryos having been formed. Those in the weak solution  $\text{NH}_4\text{Cl}$   $\frac{1}{10}$  m + sugar 0.44 m were also dead at this time. Both of these solutions exert an osmotic pressure less than that of sea-water. After forty-three hours eggs in  $\text{NH}_4\text{Cl}$   $\frac{1}{7}$  m were almost normal, and their condition in  $\text{NH}_4\text{Cl}$   $\frac{1}{10}$  m was the same, while those in the 0.44 m sugar were well up with the control. Thus again we see that the mixture exerts a far greater influence on development than either constituent acting alone is

capable of producing. It appears in this instance rather illogical to state that the extra pressure induced by the addition of sugar to the solutions of  $\text{NH}_4\text{Cl}$  caused this salt's action to become more pronounced upon the eggs, for as mentioned before the pressure of these mixtures is often below the usual pressure in which the eggs live, and from the experiment cited below we shall find that the

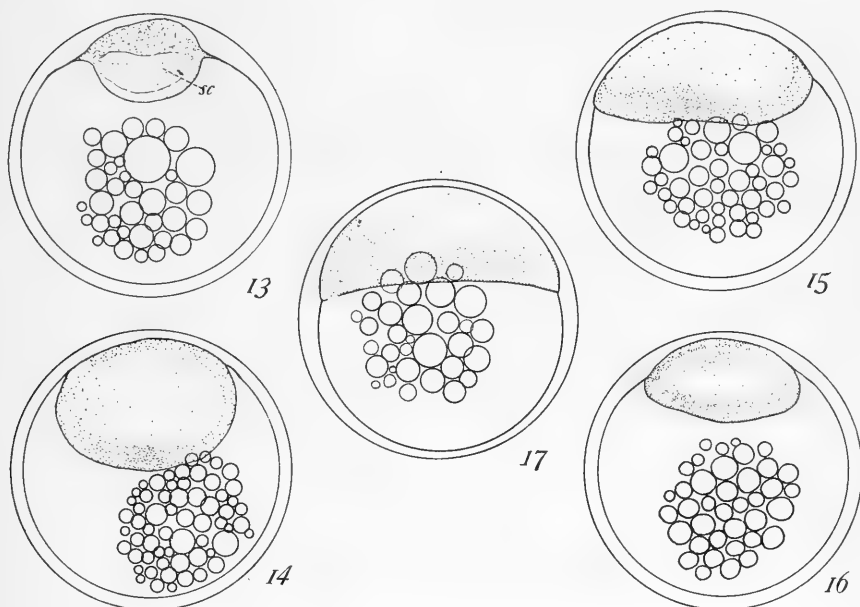


Fig. 13 An embryo when twenty hours old in  $\text{LiCl}$  0.256 m + sugar 0.293 m, the majority of eggs in this solution are in a similar condition. *sc*, segmentation cavity.

Fig. 14 The least affected egg in the above solution.

Fig. 15 The majority of the eggs in simple  $\text{LiCl}$  0.256 m solution show this condition.

Fig. 16 The most abnormal egg in the  $\text{LiCl}$  0.256 m solution at this time.

Fig. 17 A control egg when twenty hours old. All  $\times 17\frac{1}{2}$  diameters.

addition of sugar to sea-water solutions of  $\text{NH}_4\text{Cl}$ , which are of course hypertonic, furnish rather indifferent results. One might argue on the other hand that salts of the sea counteract the effects of the  $\text{NH}_4$  ion, but even if this does occur the high pressure does not particularly injure the eggs, and we are still in the dark concerning the question why the distilled water solutions of  $\text{NH}_4\text{Cl}$

act more violently in the presence of sugar unless it be due to some action which might take place when the sugar molecules split if they become inverted in the solutions. Eggs are necessarily very delicate chemical indicators and it may be that an action hitherto undetected might be shown by them. Furthermore, *Fundulus* eggs are exceptionally adapted to the study of such questions as they are not necessarily subjected to abnormally high pressure in experimentation.

Sea-water mixtures of  $\text{NH}_4\text{Cl}$   $\frac{1}{3}$  m,  $\frac{1}{5}$  m, and  $\frac{2}{13}$  m + sugar 0.293 m were used with rather indifferent results. In each of the three mixtures the yolks were slightly shrunk and in the two stronger a small per cent of the eggs always died during the first day of development, but from this time until nine or ten days old they developed in a normal manner though somewhat slower than the control. When fifteen days old in  $\text{NH}_4\text{Cl}$   $\frac{1}{3}$  m + sugar 0.293 m 95 per cent of the eggs were dead and the few embryos alive were small with feeble pulse. They appeared as embryos should when seven or eight days old. In  $\text{NH}_4\text{Cl}$   $\frac{1}{5}$  m + sugar 0.293 m 50 per cent were dead and the others were small and otherwise like those described above. The eggs in the  $\text{NH}_4\text{Cl}$   $\frac{2}{13}$  + sugar 0.293 m were all normal except for the small size of the yolks. None had hatched. In the 0.293 m sugar solution all had a normal development; and in the sea-water solutions of  $\text{NH}_4\text{Cl}$   $\frac{1}{3}$  m and  $\frac{2}{13}$  m development was almost normal except for the contraction of the yolks. The embryos were a little retarded in development and none of them hatched. These results lead also to the same general conclusion, that the *mixture* acts more violently than would either constituent acting alone, although the difference in action here is not great.

#### SUMMARY AND CONCLUSIONS

1 The membrane of the eggs of *Fundulus heteroclitus* is readily permeable to salts in solution as is shown in embryos a few days old by the fact that KCl will stop their heart action within a few moments.

During the early stages the membrane is also easily penetrated since eggs subjected to the action of strong solutions of LiCl for

one or two hours do not recover from the effects of this treatment after being returned to sea-water. Many other facts go to show the readiness with which this membrane is permeated.

2 *Fundulus* eggs develop normally, although at a somewhat faster rate, when kept on moist plates entirely out of water. The embryos developed out of water are unable to hatch while on the moist plates, but if at any time after the control has begun hatching some of the eggs are immersed in sea-water they will soon begin hatching, commencing usually in about ten minutes after being in the water and all coming out very promptly. On hatching the embryos show a positively heliotropic and a negatively geotropic reaction.

Embryos were kept for *thirty-three days*, or twenty days after the control had begun hatching, on these moist plates without beginning to hatch. The fish within the egg membrane grows in length and absorbs its yolk at about the same rate as hatched ones do. They finally die of starvation after having assimilated all of their yolk, being still confined within the egg membrane.

3 *Fundulus* eggs are not *entirely* immune to osmotic effects though it has often been stated that they are. In weak cane sugar solutions the yolks were observed to swell, this has never been seen even in eggs developing in distilled water and may probably be due to some change taking place in the sugar after it has permeated the egg membrane. In concentrated sugar solutions the yolk shrinks in a somewhat definite manner. A 1.53 m distilled water solution of cane sugar killed the eggs within twenty-three hours. The osmotic pressure of such a solution is calculated to be 34.278 atmospheres or about twelve atmospheres more than that of sea-water. Some salt solutions which exert even a greater pressure do not kill the eggs. This contradiction might be explained if the cane sugar becomes inverted in the solutions but from the evidence at hand this interpretation seems improbable. There may possibly be an action of the new substances resulting from the inversion of the cane sugar molecule which is also injurious to the eggs.

Eggs hatch in 0.166 m solutions of sugar in sea-water. On comparing the effects of sea-water solutions of sugar with distilled water solutions it was found that a pressure more than double as



high in sea-water produced a much less marked effect. Such observations seem to indicate that the eggs were less resistant to chemicals when treated in fresh water, due possibly to a slightly weakened condition when out of their usual medium. The fresh water solutions showed a strong tendency to become acid and a fungus-like growth was often present (see footnote, p. 178). It will also be recalled that the acid condition of the medium would in itself be injurious to the eggs.

4 Eggs that were subjected to the action of  $\text{LiCl}$ ,  $\text{LiNO}_3$  and  $\text{Li}_2\text{SO}_4$  were all affected in a similar manner, seeming to indicate that the cation common to the three salts was the active principle concerned. Of the large number of other salt solutions employed none of the metallic ions gave the same constant abnormalities which lithium induced. The lithium larva of *Fundulus* is as definite and well marked as those recorded by Gurwitsch and Morgan for the frog.

5 *a* It was found, as Loeb had already shown, that this egg will develop in solutions of  $\text{KCl}$  and live for several weeks without developing a heart beat. Loeb's statement that the circulatory system develops normally is incorrect, since the heart itself is abnormal, the pericardium is often greatly swollen, and other portions of the system are defective. Although eggs will live and develop in these solutions if placed in them soon after fertilization an embryo several days old will be killed in a few moments if treated in a like manner. Thus when the heart's action has once become established the embryo can no longer withstand the action of  $\text{KCl}$ .

*b* The effects of  $\text{NH}_4\text{Cl}$  on these eggs were rather general, development was retarded, the blastopore was slow closing and many short embryos resulted. The circulation was poor. Some lived in these solutions for eighteen days though none hatched.

In mixtures of  $\text{NH}_4\text{Cl}$  with  $\text{MnCl}_2$  eggs were less affected than in solutions of either of these salts used singly. This fact may be due to the antitoxic action of one cation on another as Loeb has claimed to take place.

*c*  $\text{MnCl}_2$  solutions prepared in fresh water caused a thickening or concentration of the central periblast in early stages, development was retarded, and the embryo had a feeble pulse. Some of

the embryos in the weaker solutions hatched but swam abnormally. Solutions of  $MnCl_2$  in sea-water induced similar effects.

*d* Sea-water solutions of  $MgCl_2$  caused the embryos to form one large single and almost terminal eye. This single eye results from an early fusion of the two optic vesicles. The optic cup is, therefore, abnormally large and the size of the lens in such eyes varies directly with the size of the optic cup. This condition is to be compared with that known in human monsters as Cyclopia.  $MgCl_2$ , when mixed with  $NaCl$  also caused this abnormality.

*e* Eggs that were treated with  $NaCl$  solutions showed no abnormalities during their early development. In the weaker solutions many embryos hatched but were unable to swim in a normal fashion. The  $NaCl$  affects either the nerve or muscle substance of these fish causing them to swim with jerky motions, and to fall on one side when at rest. The embryos would live for many weeks without hatching in very strong  $NaCl$  solutions.

6 Mixed solutions of salts and sugar act more violently on these eggs than either constituent would if used alone. Very small doses of a salt will give the effect of a much stronger dose, provided that sugar has been added to the solution. The presence of the sugar thus seems to augment the activity of the salt. This may be due to the additional osmotic pressure that the sugar exerts, but such an explanation is not entirely satisfactory.

Pathological Laboratory  
Cornell University Medical College  
New York City, December 1, 1906

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# MOVEMENT AND PROBLEM SOLVING IN *OPHIURA BREVISPIÑA*<sup>1</sup>

BY

O. C. GLASER

WITH FIVE FIGURES

## INTRODUCTION

The observations and experiments which I shall describe and discuss in the following pages were made in the Marine Biological Laboratory at Wood's Hole, for the purpose of testing Preyer's conclusion that ophiurans are intelligent animals. In spite of the fact that there is still much difference of opinion as to what we mean by intelligence, all will agree, I think, that it involves at least the ability to learn and to modify behavior in accordance with experience. Jennings ('06, p. 291) has formulated in the law of the resolution of physiological states, the way in which behavior is modified in experience: "The resolution of one physiological state into another becomes easier and more rapid after it has taken place a number of times." I have attacked the problem of intelligence in *Ophiura brevispina* from the point of view afforded by this law of resolution.

## PROGRESSION

Progression in ophiurans has been described by a number of observers, including Romanes ('85), Preyer ('86), von Uexküll ('05) and Grave ('00). These writers agree as regards the general method of locomotion in ophiurans, but they have not described all of the movements which these animals perform. All of these authors have noticed two types of progression, the first of which may be visualized by the aid of Fig. 1, in which the arms are numbered, and so distinguished by heaviness of line, that the most active is the widest, the least active the narrowest.

<sup>1</sup> Contributions from the Zoölogical Laboratory, University of Michigan, No. 107.

In movements conforming to type *I*, Fig. 1, *A*, the two arms 1 and 3 are used as a pair, whose strong backward stroke drives the animal in the direction indicated by the arrow. Arm 2, which projects forward rather stiffly, serves only the function of guiding, this being also the effect of 4 and 5, which are dragged behind. A slight modification of type *I, A*, is found in type *I, B*, in which the distal end of arm 2 waves from side to side, and in this manner adds to the propelling force furnished by 1 and 3. Type *I, C*, is a further modification of *I, A*, in which arm 2 instead of bending only distally makes a stroke as effective as either that of 1 or 3, and bends either to the right or to the left, so that the animal is

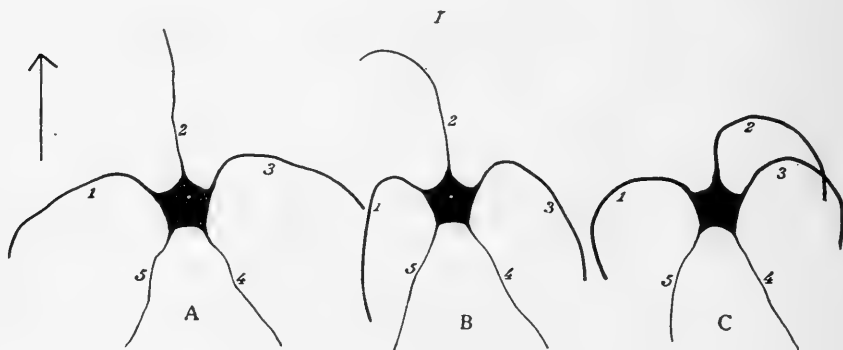


FIG. 1

propelled by two arms on one side and one on the other. The course is zigzag if regular alternations in the direction of the stroke of arm 2 occur but if this always falls on the same side the course is circular.

The movements that fall within this type are variable to an extent which has not been pointed out. *I, A*, represents in its pure forms one of the two types which all previous writers have noticed, though Grave ('00) has also observed the modification *B*, of which *C* is the extreme case. Von Uexküll ('05), who calls this type of movement *Typus Unpaar voran*, says: "Beim Bewegungstypus Unpaar voran, zeigt sich welcher grosse Unterschied in der Bewegungsamplitude des ersten und zweiten Gangpaares besteht. Letzteres verhält sich beinahe passiv. Doch kann es gele-

gentlich auch stärker in Aktion treten." Both Preyer ('86) and Grave ('00) state that the "posterior" pair is dragged behind, and I have never observed more than insignificant movements in it.

Type *II* (Fig. 2), observed by all of the writers mentioned, and called *Typus Unpaar hinten* by von Uexküll, may be described as two pairs of arms working synchronously, or alternately, the anterior pair initiating movement at one time, the posterior at another, or the movement may be begun by arms 2 and 4; by 1 and 3; by 2 and 1; or by 3 and 4; the only constant factor is the behavior of arm 5 which is invariably dragged behind.

A third type of movement, Fig. 2, *III*, not previously recorded, involves the activity of all the arms in such a manner that the animal is forced forward by three arms on one side and a pair on the other. This type may be thought of as a modification of *I, C*,

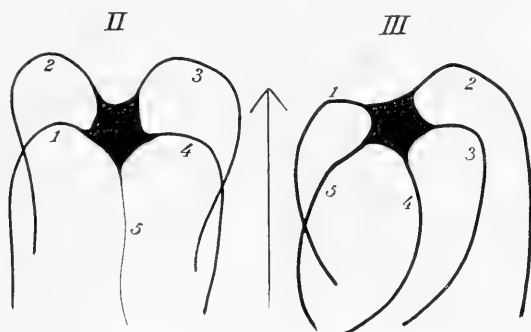


FIG. 2

in which arms 4 and 5 have become active, or as *II*, in which arm 5 has become active. Type *III*, is really *I, C*, plus an additional pair, and as in *I, C*, the course is zigzag if arm 2 alternates regularly from side to side, circular if the stroke falls always in the same direction.

It is not necessary to describe the finer variations to which these types of movements are subject; to point out, as has been done in von Uexküll's excellent paper ('05), how one may pass over into another, or how the course is affected by differences either constant or variable in the rate and strength of stroke of particular arms or particular combinations of arms. With the exception of type



Unpaar voran, in which according to von Uexküll effective movements occur in the two arms which are usually dragged passively behind, I have observed that *Ophiura brevispina* moves in practically all the ways in which it is possible for a pentaradiate animal of its construction to move.

#### INDIVIDUALITY

The movements described are directly dependent upon the pentaradiate symmetry, but this symmetry does not exhaust the possibilities of behavior. A little observation shows that each animal is unique at any given time and that while its movements fall within the system of classification proposed, they have peculiarities that distinguish them from other movements of the same type.

In general the movements may be either rapid or slow, and certain individuals seem on first acquaintance to be distinctly active or distinctly sluggish. More careful study shows, however, that very sudden changes of behavior occur, and that an active, rapidly moving animal may unexpectedly enter into a state of sluggishness that sometimes lasts for hours. I do not understand these sudden changes. They are not due to the conditions in the aquaria; they occur with great suddenness and not in all of the animals; they are not due to either gentleness or roughness in handling because either may or may not be followed by a change in the behavior of the same individual in successive trials. Possibly any sort of handling may, in certain physiological states, cause a change of behavior, but what the physiological state in which this occurs is, is hard to ascertain. In certain experiments in which I encumbered the arms with rubber tubes, after the manner of Preyer ('86), I frequently encountered the same sudden change from activity to passivity, and arms which were flexible and easily encumbered, would suddenly bend at their tips and stiffen, so that it was impossible to slip the tube over them. This stiffening might take place at the first trial, or some other one, and never again, or it might reoccur upon every attempt to encumber the arm.

Periodic changes from activity to sluggishness also occur.

Thus, in June of the present year, more than half of the animals I studied were very active and quickly responsive to stimuli during sometime of my acquaintance with them, but by August the whole race had changed. Perfectly fresh material brought into the laboratory in excellent condition and kept in large tanks of running sea-water, was so sluggish that I was forced to give up the experiments which I had planned for that month. None of the stimuli employed in June elicited reaction, and acids sufficiently concentrated to attack the skeleton, as well as the electrical current, resulted in nothing but a few spasmodic contractions with no attempt at progression or escape. What the reason for this change was is not certain. A sluggish individual almost always has very large bursal openings; in fact, it is possible to predict with considerable certainty the behavior of an individual by examining its ventral surface. The enlarged bursal openings may be consequences of the spawning process, and the periodic change of behavior of the breeding activities. *O. brevispina* begins breeding in June and ends in August. Late in June many individuals have spawned, and many have the enlarged bursal openings; by the middle of August all have spawned (Grave '00) and most of the individuals have the enlarged bursal openings. As the genital ducts lead into the bursæ—which in some species are used as brood-pouches—their enlargement may very well be due to sexual activity, which is a drain upon the animals, and undoubtedly leaves them in a state of physiological depression. If this view is correct, the enlarged bursal openings are the indices of a lethargic state following the breeding season.

Rapidity and sluggishness of movement have consequences of great importance in problem solving. Sluggish animals not only make fewer movements and take more time to perform them than active individuals, but they use in general fewer arms; their movements are less varied, and the arms very rarely come into contact with one another or cross. All this is very different with active individuals; their movements are quick and varied; they use relatively more arms, often move these through greater arcs than the sluggish animals; and, in addition, the arms touch and cross with great frequency. How "contacts" and "crosses" are related

to activity and sluggishness is easy to see. An active individual using four arms in progression has a much greater opportunity to make "crosses" and "contacts" than if fewer arms were used. Very often when the animals move by means of two pairs of arms, the anterior pair is crossed by the posterior regularly. The same frequently happens when only three arms are used.

Contacts and crosses also depend on the length of the arms, as the chances that they will occur in long armed individuals are greater than in short. How important arm length is, is indicated in the following table in which are summarized observations on three individuals which were active, but differed in the lengths of their arms and also in the manner of using them. The effect of the latter factor emphasizes that of the former. The longest armed individual *A* used the "two pair of arms" stroke, only once in a total of 141 effective backward strokes, whereas the shorter armed individuals *B* and *C*, used this stroke eight times in 129 and four times in 126, respectively.

TABLE I

Individual	No. of Movements	Per cent Contacts	Per cent Crosses	No. of arms moved					
				1	2	3	4	5	
A	141	13	119	1	26	28	1	0	times moved
B	129	11	16	0	29	13	8	0	times moved
C	126	6	2	0	16	26	4	0	times moved

#### RIGHTING MOVEMENTS

Two types of righting movements were observed, only the first of which has been described by von Uexküll ('05) in an excellent paper illustrated by means of kinoscope photographs, and by Grave ('00), who says: "Two adjacent arms straighten out so that together they form a straight line. On these arms as an axis the body revolves, being pushed over by the three remaining arms, but mostly by the median one of the three."

This description, correct as far as it goes, is incomplete. At the bases of the straightened arms, and in the interradian portion of

the disc between them, movements occur whose effect is to bend the ventral surface in the direction indicated by the arrows. When this process, by which a small portion of the ventral surface is brought into the normal position (Fig. 3, *A*), has proceeded far

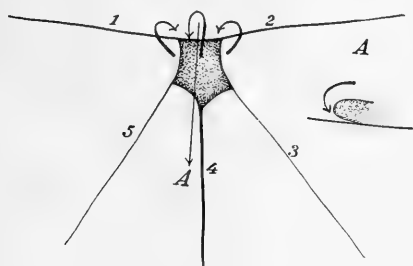


FIG. 3

enough, the animal is righted suddenly by its own weight, since while the process described has been going on, arms 3, 4 and 5 have so elevated the dorsal surface of the disc that this falls into the normal position.

In the second type of righting movement, Fig. 4, arm 2 curves near its base, and bends

under the disc which, as in the previous case, is elevated by the other arms, particularly by 4 opposite 2. The disc thus rotates on the base of 2 as a pivot, and after it has been sufficiently elevated, the animal falls into the righted position of its own weight.

The length of time required to execute the righting reaction was measured on eight individuals. I have summarized these results in Table II, in which are given the average time for each individual, as well as the maximum and minimum consumed. (See Table II.)

These averages of course do not show the differences between the successive individual rightings of any of the animals used. These differences were in some instances very large, and have had a great effect on the averages. (See Table III.)

These measurements show that the variations from the mean may be very great; that because an individual has righted itself very quickly a number of times is no reason for believing that it will continue to do so. in spite of those cases in which righting took place slowly, the

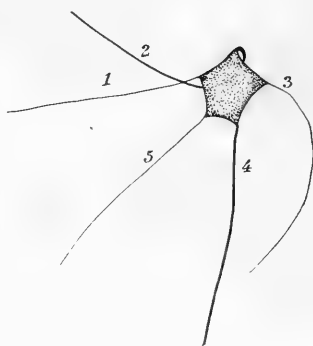


FIG. 4

TABLE II  
Average righting-times of eight animals

Date	Intervals	No. Trials	A	B	C	D	E	F	G	H
June 27	0	8	3.62"	3.62"	14.50"	4.12"				
27	5'	8	2.86	4.37	25.50	5.12				
27	3 hrs.	10	4.20	3.80	10.60	4.10	4.00"	16.20"	4.90"	3.60"
28	16 hrs.	10	5.10	5.60	6.40	43.30	5.10	10.30	5.10	4.40
30	64 hrs.	10		3.80	15.60	21.60	29.10	7.10	3.50	7.9
	.		Max. Min.	Max. Min.	Max. Min.	Max. Min.	Max. Min.	Max. Min.	Max. Min.	Max. Min.
			8" 2"	14" 2"	43" 4"	198" 2"	89" 3"	39" 3"	12" 2"	24" 2"

TABLE III

*Individual righting-times of eight animals in many successful trials*

A	B	C	D	E	F	G	H
3	5	7	2	4	8	5	3
4	3	5	5	3	12	5	4
3	3	12	4	3	9	6	3
2	2	7	3	4	17	4	3
5	5	20	6	4	12	5	2
3	3	12	4	3	17	4	3
3	5	34	4	5	13	3	3
7	3	19	5	4	39	2	7
3	4	19	2	4	22	3	3
3	2	31	3	6	13	12	5
3	7	8	6	4	9	4	4
3	5	28	4	3	5	10	4
3	4	43	8	5	3	4	4
3	4	33	4	4	15	6	3
3	4	14	4	6	7	6	8
2	5	28	10	4	4	4	4
4	3	10	3	4	14	4	4
3	4	8	4	7	11	5	3
4	3	23	3	7	24	4	5
3	3	6	3	7	11	4	5
3	4	10	3	4	6	3	5
3	4	12	5	29	4	2	7
8	4	8	3	31	3	4	5
7	5	17	4	35	7	3	3
3	4	7	4	12	8	3	24
4	4	5	9	7	6	6	6
3	3	4	29	37	10	3	5
4	4	6	23	38	3	2	6
7	7	5	15	89	15	4	9
7	4	5	20	9	9	5	9
5	4	4	29				
6	5	6	40				
7	14	6	18				
5	6	9	198				
3	6	5	7				
4	3	14	5				
	3	8	4				
	5	5	11				
	4	27	25				
	4	6	45				
	3	21	11				
	5	27	12				
	4	12	14				
	4	20	14				
	3	24	41				
	3	6	39				

records when averaged show that these animals, on the whole, may be expected to right themselves in less than 45 seconds. One fact of considerable interest is clearly demonstrated by the averages as well as by the individual records—there is no reduction in the amount of time required to perform the righting act; in other words, under normal conditions, these animals do not improve by practice in the execution of their righting movements.

#### PROBLEM SOLVING

The expression "problem solving" is almost self-explanatory. Under this heading, I have placed such behavior as an ophiuran exhibited when stimulated by interference more or less unusual, and from which it was able sooner or later to escape. What I did was to observe the way in which the escape was made—the problem solved—and how much time was consumed in doing it.

The problem—the same as that employed by Preyer—was to rid one or more arms of the small pieces of loosely fitting rubber tubing with which I encumbered them. In the selection of individuals for experiment, my choice was guided by two considerations:

whether all the arms were approximately equal in length, neither broken, nor recently regenerated; and whether the individuals were not too active to make the observations easy to record.

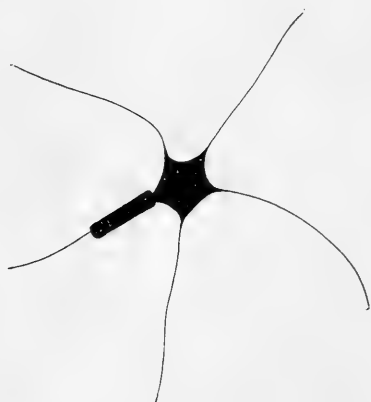


FIG. 5

When encumbered in the manner represented in Fig. 5, an ophiuran does many things, some of which are recorded in von Uexküll's photographs. At first it may pass through a brief latent period, during which it lies motionless on the bottom of the dish, and then it may crawl, dragging the

encumbered arm behind it. Often the animal moves at an angle to the encumbered arm, or in rare cases in the direction of it. The

progression may be of a very violent character involving many contacts and much crossing of arms, or the animal may simply writhe, without changing its location. If it does not move about, it usually waves one of its arms, especially the encumbered, in a horizontal plane, though the movements may also occur in a vertical plane and in circles. The encumbered arm is moved in a vertical plane oftener than the unencumbered ones; is frequently rubbed against the disc; against the adjacent arms; against the sides of the dish; and even against itself. Sometimes the encumbered arm is waved over the disc, much as a man waves a long whip, and then is "cracked," so that the encumbering tube moves nearer the distal end, and often slides off. When relieved the animal usually does not remain quiet, but continues its movements for a short time and makes several strokes that remove it from the place where the tube was gotten rid of. If at the instant of riddance the animal was not progressing, a short journey is begun at the moment of relief.

When encumbered on more than one arm, the latent period is longer than when only one arm is encumbered; the first movements are not through as great arcs, nor are they so long continued in any direction. One movement is succeeded rapidly, not by its duplicate, but by another in a different direction, and this by still another. The behavior changes constantly.

If all of the arms are encumbered, the above changes in behavior cease very soon, and an entirely different kind of action is begun. Instead of movements in the usual sweeping manner, the arms quiver and tremble. In one case, one arm (the first to be rid of its rubber tube) in particular attracted my attention by quivering when the rest of the animal was perfectly quiet. These quivering movements occur in a horizontal plane, and are so rapid, and many of them so slight, that it is impossible to record them accurately without special apparatus.

Of all these movements, several are more effective than many others in bringing about riddance. The most effective are the "whip movement," the "stripping movements;" certain of the "wavings," and violent progression which involves a number of different movements. Of these the whip movement is the rarest;



the violent progression next, whereas the strippings and the wavings are the commonest of all.

These observations open two ways in which the problem of resolution may be attacked; by studying the time taken to solve the problem and by noting the relative frequency of the most effective movements. The time and the frequency might both remain constant, or might change, or only one might change. As a reduction in the amount of time taken to solve the problem need not necessarily be due to an increase in the relative frequency of the most effective strokes, these two must be considered separately, although an increased frequency of strokes best fitted to solve the problem would involve a reduction in the amount of time. If a reduction in the amount of time required does occur, it means that the physiological state produced by the rubber tube has been resolved into the normal state more rapidly than it was resolved the first time. In other words, the animal has learned by experience.

The following Table IV contains my measurements of problem solving time. In every case the animal was given the same problem consecutively, viz: the same rubber tube was placed on the same arm, under the same conditions. As little time as possible was lost between trials.

TABLE IV\*

Trials

Individual.....	1	2	3	4	5	6	7
A	3' 45"	5' 00"	2' 00"	3' 00"	6' 00"		
B	0' 39"	4' 30"	1' 00"	1' 1'	4' 00"		
C	0' 45"	1' 45"	0' 1"	5' 00"	1' 30"		
D	2' 00"	3' 00"	7' 30"	3' 00"	3' 00"	2' 15"	3' 30"
E	1' 30"	0' 45"	1' 30"	1' 40"	5' 15"	3' 30"	

\*These measurements include the latent periods.

The number of trials recorded in Table IV is small. I was prevented from collecting more data by the sudden changes of behavior before alluded to. Other animals were tried but failed to react regularly even five times. The results as they stand, however, are worthy of confidence; they are representative of the whole behavior which is varied and uneven; like the measurements of

righting time they neither increase nor decrease—the apparent increase being due to the failure to respond, for had this failure occurred sooner, some of the last measurements would have been smaller than the first. Fatigue played no part in the result, as the figures are too uneven.

The objection might be advanced that these cases which I have called “problems,” were not such; that there was no reason why the animals should modify their behavior, and that what they did under the conditions of the experiment was nothing that they would not have done under normal conditions. This objection is met satisfactorily I believe by the following experiments.

A given arm was stimulated by encumbering it with a rubber tube, or by painting it with strong or dilute formalin or hydrochloric acid of different strengths. These trials, of which I made a great many, yielded very definite results. In only one case did an animal progress in the direction of the stimulated arm; in a few cases at an angle to it, using it as one of the propellers, whereas in the vast majority of cases it moved in the direction diametrically opposite the stimulated arm. If the stimulus was strong, the movements were very violent, but no difference in direction was noted in the case of weak and strong stimuli. Under ordinary circumstances it is impossible to predict the direction in which an ophiuran, all of whose arms are of the same size, will move, but if one of the arms be encumbered the prediction that the animal will move away from the stimulus will be verified in the vast majority of cases. I think it is justifiable to assert that the direction of progression has been determined in these cases, and if this is true there is a determining cause—a problem.

My second line of inquiry—whether encumbered animals showed a noticeable increase in the number of movements best adapted to solve the particular problem given, was begun by finding the percentage of crosses and contacts in the same animals under the two conditions stated. The results are summarized in Table V.

As contacts and crosses usually result from wavings I counted these in animal *II* unencumbered and with one arm encumbered. The results are summarized in Table VI

The general conclusion to be drawn from these experiments is that there is neither a decrease in the amount of time taken to solve the problem, nor an increase in the relative frequency of movements best fitted to solve it. In other words, the animals did not modify their behavior in accordance with the law of resolution, and consequently, so far as is objectively recognizable, learned nothing.

TABLE V

Animal	Arms Encumbered	Movements	Per cent Contacts	Per cent Crosses	Problems
I	0	231	4.0	2.0	0
I	1	202	3.9	1.9	3
II	0	267	13.8	10.8	0
II	5	553	9.2	11.2	5

TABLE VI

Animal	Arms Encumbered	Movements	Per cent Wavings
II	0	117	26.4
II	1	192	19.3

## DISCUSSION

The facts which I have brought forward in the foregoing pages agree with those of Preyer and von Uexküll in showing that in problem solving the animal repeatedly changes its behavior, not persisting in a certain reaction when that is unsuccessful. If I venture to take issue with Preyer, and to assert that the behavior which both he and I observed does not warrant the conclusion that ophiurans are intelligent, I must rest my claim upon the validity of my interpretation of the facts, and this validity I shall now attempt to establish.

The behavior of *Ophiura brevispina* may be summarized by saying that this animal under normal conditions performs practically all the movements possible to a creature constructed as it is; that except for this limitation, its ordinary behavior is not predictable,

and that even the righting movements, because of their variety occupy a place between the ordinary behavior and reflex behavior, for though more definite than the former, they are less precise than those highly perfected types of response which gave us our first idea of reflex action.

Regarding the manner in which ophiurans rid their arms of encumbrances, Preyer ('86, p. 125) says: "Aus den beschriebenen und ähnlich leicht zu variirenden Versuchen ergibt sich zunächst, dass Ophiuren in 5-fach. verschiedener Weise sich gegen die beim Tasten und kriechen ihnen sehr hinderliche Bekleidung mit einem Schlauche vertheidigen: (1) streifen sie ihn ab durch Reibung am Boden wenn er locker ist, (2) schleudern sie ihn fort durch geissel. förmiges Hin und Herwerfen, (3) drücken sie ihn fest gegen den Boden mit dem freien Nachbararm, und ziehen den Arm aus dem dadurch fixirten Rohre heraus, (4) stemmen sie abwechselnd beide Nachbararme mit deren Zähnchen unten gegen dasselbe und schieben ihn ruckweise ab, (5) brechen sie durch Selbst-amputation den Arm mit der unbequemen Bekleidung ab. Hilft dass eine Verfahren nicht, dann wird das andere angewendet. Sehe ich hier von dem letzten, der Autotomie, ab, von der noch die Rede sein wird, so beweist schon die 4-fache Art der Abwehr bei einem und demselben Individuum unter denselben äusseren Verhältnissen, dass hier kein einfacher Reflex vorliegt. Vielmehr besitzen die Ophiuren die Fähigkeit sich ganz neuen, von ihnen noch niemals erlebten Situationen schnell anzupassen."

"Wenn Intelligenz auf dem Vermögen beruht, Erfahrungen zu machen, d. h. zu lernen, und das Erlernte in neuer Weise zweckmässig zu verwerthen, so müssen also die Ophiuren sehr intelligent sein."

Preyer's reasoning seems to be this: When encumbered on its arms the animal moves in different ways; failing to free its arms by these movements, it moves in other ways, and continues to change its movements until the encumbrances have been removed. The animal thus exhibits the process of discovery by elimination, learning in other words, and is therefore intelligent.

If this indeed be learning, then all movements which any organism may under any circumstances execute are outward signs

of the process, for movements are never without cause, and the stimulus is aggravated, alleviated, or unchanged by them. Whatever be the result of the movement, the animal "learns" what has been the effect upon the stimulus, the cause of the movement. Two criticisms may be made of this point of view: In the first place, in behavior such as that of an ophiuran, movements which fail to solve a specific problem, or to contribute anything whatever to its solution, are often repeated immediately. If the animal learned anything from them, it forgot what it learned at the instant of learning, for the intervals between two successive movements which fail for the same reason may be less than one second; to forget as rapidly as to learn, can be objectively recognized as neither. In the second place, in ophiurans at least, it is the exception for an animal to perform only one movement at a time. Usually a considerable number, four, five, or six distinct movements are performed synchronously. All of these, on the assumption I am criticising, result in learning, but the knowledge which they give may be of two sorts; some of the movements may tell the animal how to solve the problem, the others, how it cannot be solved. It is impossible for me to believe, without striking evidence to the contrary, that an ophiuran can learn at the same instant half a dozen facts, belonging some to one, some to the other of two distinct categories.

If the idea that mere movement in various directions is a sign of learning, involves the serious difficulties which it seems to me to involve, we have nothing but behavior more or less permanently modified as the result of experience to fall back upon. I have shown that under ordinary circumstances *Ophiura brevispina* does not improve with practice, in its righting behavior, and in problem solving it shows no greater aptitude. I am, therefore, forced to the conclusion that neither intelligence nor even learning have as yet been demonstrated in this animal.

My experience with ophiurans also leads me to the conclusion that resolution will be very difficult to demonstrate, not only because of those sudden changes in behavior for which it is difficult to assign causes, but also because of the remarkable "action system" exhibited by these animals. This action system shows better

than many others, that behavior is structure in motion, and that complexity of behavior depends on the complexity of that which behaves. An act performed by one arm may also be performed by any of the others. The arms may all do the same thing at the same time; some may do one thing and others another; and finally a single arm may execute different movements at different levels. As the disc itself may also execute varied movements, the number of possibilities is enormous. With this marked versatility to contend with, it is not surprising that resolution, demonstrated according to Jennings ('04, '05, '06) for Protozoa, Cœlenterates, and other forms lower in the scale of complexity than echinoderms, or as low, should remain undemonstrated for ophiurans. The number of movements possible to an ophiuran is immense; if the animal only acts, the chances that it will perform movements fitted to relieve a certain physiological state are better than the chances that such will be the case in most other animals. If one of the many movements that will serve is not performed, another will be, and we should not expect to find resolution, unless the fit things to be done are few. Any of the problems presented might have been solved in a variety of ways. One or more of these ways were superior to any of the others, but all served the purpose. Where the variety of solutions to a problem is great, there is no need of resolution, and it does not occur.

I have profited much by the elaborate criticism which Professor Jennings made of an earlier draft of this paper, and I take this occasion to thank him for his kindness.

University of Michigan  
Ann Arbor, Mich.  
February 1, 1907

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# OCCURRENCE OF A SPORT IN MELASOMA (LINA) SCRIPTA AND ITS BEHAVIOR IN HEREDITY

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WITH ONE PLATE

During the year 1904, early in the breeding season of the chrysomelid beetle, *Melasoma scripta*,<sup>1</sup> about 1000 pupæ, and larvæ, in advanced stage, were collected from willows in the neighborhood of an artificial lake near Stanford University.

Such of these as were not parasitized matured during the latter part of April and early May. The adults represented the dichromatic extremes of the species, the elytra being either spotted-brown (referred to in this, as in previous papers, as "S"), or black (referred to as "B"), the thorax in each case having a central black area widely emarginated with brick red. In the center of each red area and nearly adjacent to the central black region (sometimes approximating it) is a small black spot representing a single punctuation. (Figs. 1 and 2.)

During the course of breeding through four generations from this collected material there occurred a number (four or five) wholly black individuals (Fig. 3), thorax as well as wing covers being totally black (referred to in this paper as "AB"). Since during the casual outdoor observations made throughout that

<sup>1</sup>A description of this beetle is given in the *Journal of Experimental Zoölogy*, 1905, vol. ii, pp. 117, 136, and vol. iii, pp. 320-336, where it is called *Lina lapponica*. It seems that this identification made for me is not correct. The beetle is evidently the one figured by Riley under the name *Plagiodera scripta* (Fabr.) *Ann. Rept. Agric.* for 1884, pp. 336-340, pl. viii, Figs. 1 and 2; by Lintner, under the name *Lina scripta* (Fabr.), *Rept. N. Y. State Entomologist* for 1895, pp. 181-189; and by Felt as *Melasoma scripta* (Fabr.) in *N. Y. State Museum, Memoir* 8, vol. i, pp. 317-322, Pl. 16, Figs. 16-20.



season no such freaks were found, and those bred in the laboratory failed to mate, they were looked upon as representing possibly a pathological condition.

However, in 1905 outdoor scriptas were kept under constant surveillance throughout the breeding season. At stated periods, four or five weeks apart, several hours were spent in the field, at which time several hundred individuals passed under inspection with the following result:

First inspection, March 4. Thousands of beetles in a limited area feeding and beginning to breed. (These were in all probability the hibernated individuals from the previous year.) No "all black" (AB) individuals were observed. Several hundred individuals, representing each of the dichromatic extremes, were collected at this time for indoor controlled breeding.

April 12: Many thousands of beetles observed; two AB females collected.

May 14: Many thousands observed, two AB females collected.

June 21: Many thousands observed, two AB males collected.

July 28: Individuals in this particular feeding ground becoming noticeably fewer. Many hundreds of beetles observed, one AB female collected.

August 21: Many hundreds of beetles observed, one AB male collected.

Hence a total of five females and three males were collected in this locality during the five months the locality was under observation and covering the breeding season of the beetle. Three similar sports were collected during this time from poplar trees a half mile or so distant from this locality.

During the progress of these outdoor inspections, indoor breeding was in progress from the collection of March 4, 1905, that is, a collection made up of "spotted" (S) (Fig. 1) and "black" (B) (Fig. 2) but no "all black" (AB).

Four generations were reared to maturity from this collection. The following table gives the data in regard to the occurrence of the sport AB with the character of the lineage in each generation. (The term "sport" is here used in the sense of a singular and decided variance from the normal type.)

There occurred, therefore, in the breeding room, a total of 20 AB, or sport individuals in a total of 11,369 individuals reared during the breeding season, most of these coming from the immediate collection of March 4.

Inspection of the table shows that in the first generation, 168 matings were made, one brood only being reared from each pair. Parentage was represented by both  $S \times S$  and  $B \times B$  matings. The sport AB was found in the progeny of each series, ten in the former, six in the latter. In other generations matings were made in the S line only. In the second and fourth generations, single broods only were reared from each pair, as in the first generation,

TABLE I

	GG Grand- parents.	GGrand- parents.	Grand- parents.	Parents.	No. Matings made.	No. broods reared.	Total Indv.	No. AB ♂   ♀	Total AB
1st gen.				$S \times S$ $B \times B$	119 49	119 49	5034	8 2 1 5	16
2d gen.			$S \times S$	$S \times S$	45	45	1050	1 0	1
3d gen.		$S \times S$	$S \times S$	$S \times S$	32	180	4736	3 0	3
4th gen.	$S \times S$	$S \times S$	$S \times S$	$S \times S$	19	19	549	0 0	0
Total.....					264	412	11,369		20

while in the third generation, an average of five or six broods were reared from each pair (a minimum of two, a maximum of fifteen broods). With three exceptions, not more than one AB individual occurred in any one brood. In the first exception two individuals (a male and a female), occurred in a single brood, in the second exception five males occurred similarly, and in the third exception two males. We find, therefore, in a total of 264 matings, fourteen only producing sports.

That the occurrence of the sport was normal, that is, was not due to laboratory conditions, is evidenced by the fact that but comparatively few sports occurred (20 out of a total of 11,369 individuals.) Since all broods in the breeding room are under practically the same conditions, had an extrinsic influence been at work

tending to produce this variant, certainly more individuals would have shown the effect.

The numerical results in second, third and fourth generations of the occurrence of the sport might have been different had continued breeding of the progeny of the first B parents been carried on and if a larger number of broods had been reared from each pair.

There was found to be no greater likelihood of the recurrence of a sport in a brood from parents in whose lineage sports had previously occurred than in broods from parents in whose lineage no such sports were known to have occurred in so far as this point was tested. Numerous matings were made between individuals having AB sisters or brothers, but in no case was there a recurrence of the AB character.

That the sport is of occasional occurrence in the field has already been noted. The stable character of many sports or aberrant variations in animals such as this appears to be is undoubted, since it has been shown several times, notably in the race of Ancon sheep from a single short-legged, long-backed ram, in 1791, and the production of the Mauchamp-merino breed of sheep in 1828 from a single ram with long, smooth, silky wool.<sup>2</sup> Also more recently the production of a race of polled Hereford cattle in Kansas in 1889 from a single polled bull.<sup>3</sup>

The main purpose of the present investigation was to determine, in case the new character should be found to be stable, its hereditary value in relation to the characters of the parent species. The hereditary value of each of the dichromatic extremes with relation to the alternative extreme had been previously determined.<sup>4</sup>

To test the hereditary value of the Melasoma sport, the first two sports occurring simultaneously in the first generation were used as parents for succeeding generations. Other sports were mated with the parent forms.

<sup>2</sup>Darwin, 1868, *Animals and Plants*, vol. i, p. 126.

<sup>3</sup>Guthrie, 1906, *Proc. Amer. Breeders Assoc.*, vol. ii, p. 93.

<sup>4</sup>McCracken, 1906, *Inheritance of Dichromatism in Lina lapponica*. *The Journal of Experimental Zoölogy*, vol. iii, pp. 320, 336.

In the first matings of  $AB \times AB$ , the male parent was of S parentage, the female of B parentage. Five broods were obtained from this mating, a total of 130 individuals. Of these 77 individuals were similar to the immediate parents, that is, were AB in character, and 42 individuals were similar to the grandparents on the female side, that is, were B in character. In eleven individuals the wing covers were black, while the character of the thorax was a mosaic of the thorax of AB and B (Plate, Fig. 4); that is, the emarginate area was in part black and in part red. The black blotch upon the red might be in any position, covering the anterior half, the posterior half or the median two-thirds, as indicated in the figure. We find here, therefore, a series of variations arising in the progeny of a mating between two similar sports or aberrant variations, so that if the latter had not arisen, first, it would have been considered but an "extreme variation" in a series. No such mosaic was observed in field collections. There was no recurrence of the "S" type. The sport or parent character predominates somewhat in the offspring over the B type (1.8 : 1).

The mosaic type appears to be a heterozygous form as in matings of this type (I) the offspring always revert to the AB or B type with only an occasional I individual.

With the 77 AB, the 42 B, and the 11 I individuals thus obtained, six categories of matings were made as shown in the following diagrams. These diagrams show the pedigree for three generations and the character of the brood produced in each.

DIAGRAM I—*Mating Category a*

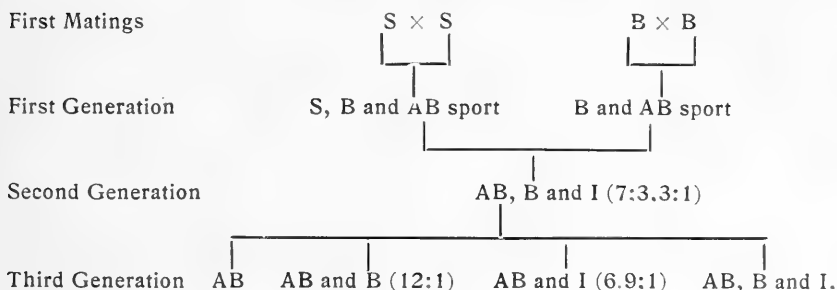


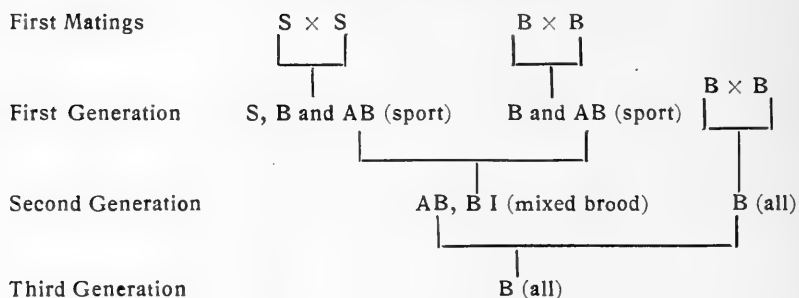
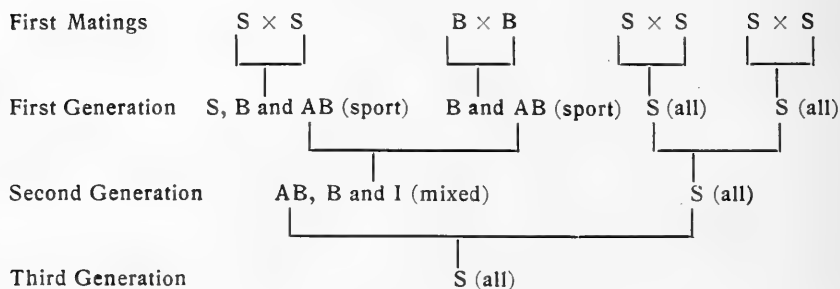
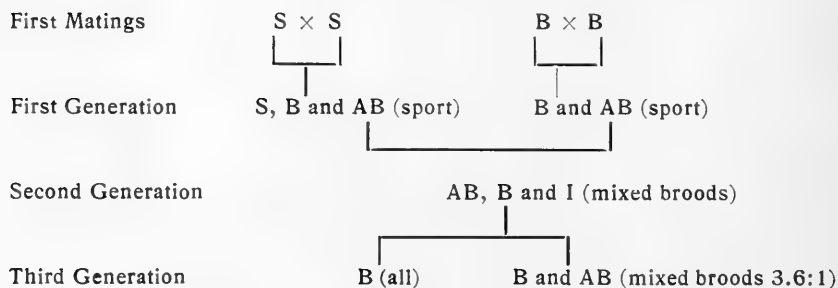
DIAGRAM II—*Mating Category b*DIAGRAM III—*Mating Category c*DIAGRAM IV—*Mating Category d*

DIAGRAM V—*Mating Category e*

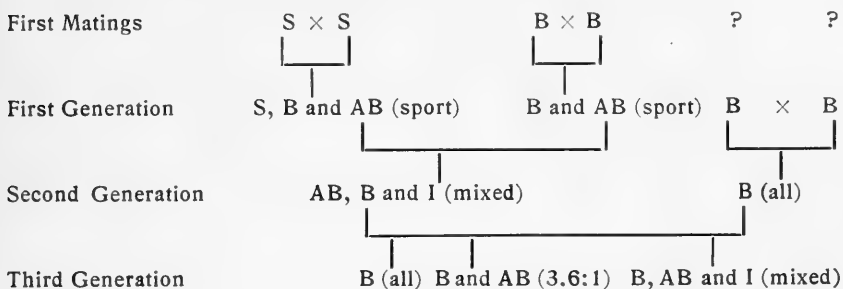
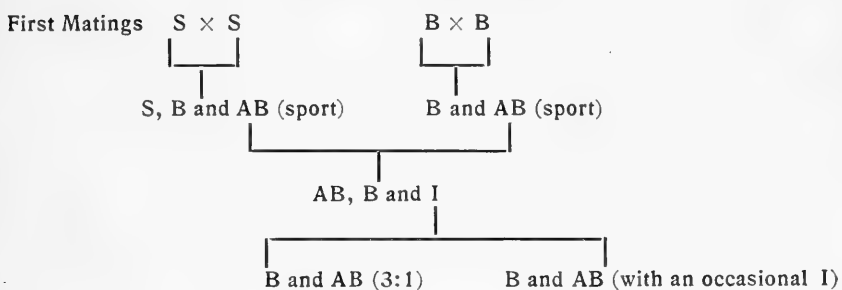


DIAGRAM VI—*Mating Category f*



The following table gives the results in total as to the character of the individuals reared in the third generation in the different categories, from the first matings, the lineage for which is given in these diagrams.

In the papers previously referred to on "heredity of dichromatism," it was shown that the color type S is dominant over the color type B, not at first completely so in every case, but showing from generation to generation an increasing prepotency in that direction. B was considered recessive in that it disappeared wholly or partially in the first generation of a cross between S and B, but bred true from the time of its reappearance in the second generation. Because B in its ontogeny passes through the S stage, it was suggested ('05) that S represents possibly the older, B the newer type. AB in its ontogeny passes through the S and B stages. Its normal infrequency of occurrence makes it appear

to be either the newest type or an atavistic form. Upon the assumption that it is a new type, we would expect its behavior to S and to B to parallel that of B to S. Our expectation is in the main fulfilled. Inspection of Table II shows that in the progeny of  $AB \times B$  (category *b*) all the offspring are B. In the progeny of

TABLE II (Third Generation)

Mating Category	Parents	Tot. No. Broods	Char. of Broods	Tot. No. Indv.	Tot. AB	Tot. B	Tot. I	Ratio of AB, B or AB:I
<i>a</i>	$AB \times AB$	61	S ..... 0 B ..... 0 AB (all) . . 24 AB & B . . 6 AB & I . . 27 AB, B & I 4	586 133 651 105	123 569 56	10 82 31	18	12:1 6.9:1 5.6:3.1:1.8
<i>b</i>	$AB \times B$	5	B ..... 5	135 (all B)	0	0	0	
<i>c</i>	$AB \times S$	17	S ..... 17	448 (all S)	0	0	0	
<i>d</i>	$B \times B$ (both parents from AB)	16	S ..... 0 B (all) . . 2 AB & B . . 14	52 498	0 108	52 390	0	1:3.6
<i>e</i>	$B \times B$ (one parent only from AB)	39	S ..... 0 B (all) . . 14 AB & B . . 23 AB, B & I 1 B & I . . . 1	381 629 17 30	381 139 1 29	381 490 15 1	1	1:3.6
<i>f</i>	$I \times I$ (both parents from AB.)	4	S ..... 0 AB(all) . . 0 B(all) . . . 0 AB & B . . 3 AB, B & I 1	80 22	20 4	60 15	3	1:3

$AB \times S$  (category *c*) all the offspring are S. That is, both S and B dominate AB completely in crosses between S and AB or B and AB. In these two categories AB is completely recessive, in the sense that it does not appear in the soma of any of the offspring.

In  $B \times B$  matings, extracted B (category *d*, Table II), both parents having been born of AB parents, two kinds of broods are

produced; that is, broods in which all the individuals are B and mixed broods of B and AB, individuals of B character predominating. In these mixed broods the proportion of B to AB is 3.6 to 1. This parallels the history of the behavior of S toward B in matings of dominant S hybrids as previously determined.

In  $B \times B$  matings (category *e*, Table II), in which one of the parents was born of AB parents, two kinds of broods also appear; that is, broods in which all the individuals are B, and mixed broods of B and AB, or B, AB and I or B and I, the character B predominating. It is noticeable that in this case, in which one-half as many AB ancestors are represented, the proportion of wholly B broods exceed that of the former cross by about 3 to 1, while the proportion of B to AB in mixed broods remains the same, 3.6 to 1. In neither case were any broods produced that were wholly AB in character.

In  $I \times I$  matings (category *f*, Table II) mixed broods result in which B is the predominating character, AB taking a second rank and I recurring but rarely, thus showing the heterozygous character of I.

The hereditary value of the character B in the first generation of a cross between B and AB (that is, B not previously contaminated by AB) appears from these data to be, therefore, equivalent to the hereditary value of pure S to the character B. The hereditary value of pure S and pure B with reference to AB have the same equivalency.

The stability of the sport character AB is absolute in neither first nor second generation matings, but comparison of data of first generation mating (Diagram I, category *a*) with Table II, *a*, shows an increased preponderance of the sport character in the latter case. AB in the second generation breeds true in more than two-thirds of all broods produced and in all mixed broods greatly predominates in the offspring. It is again noticeable that no S individuals appear in the offspring, although reference to Diagram I shows that as many S as B individuals are represented in the ancestry and reference to Table I shows AB appearing in broods of  $S \times S$  parentage as well as those of  $B \times B$  parentage. This suggests that the type AB is after all but a new phase of the type B



and that its appearance in the progeny of  $S \times S$  is coincident with the appearance of B in the progeny of  $S \times S$ . Its failure to transmit S while transmitting B is in harmony with this interpretation.

The character AB therefore answers to the requirements of Mendelian recessiveness with respect to its behavior toward the alternate characters, S and B; that is, it disappears in a first cross. Since the matings in category *a* represent merely second generation matings from the sport AB, the data here cannot be consistently compared with the data from matings of true recessives. Behavior of AB as a recessive was tested later in the season with extracted AB from hybrids S.

For fourth generation data, matings were made in the following categories:

Category *a*.  $AB \times AB$ , both parents of AB ancestry, for two generations; that is, second generation from sport parents (Diagram I).

Category *b*.  $B \times B$ , both parents being wholly of AB ancestry, for two generations (Diagram I).

Category *c*.  $S \times S$ , both parents being hybrids from  $S \times AB$  (Diagram III).

Category *d*.  $S \times AB$ , the S parent being of pure S ancestry for two generations, the other of AB ancestry for two generations.

The following table gives the results of these matings:

TABLE III (Fourth Generation)

Mating Category	Grand-parents	Parents	Tot.No. Broods	Character of Broods	Tot. No. Indiv	Tot. AB	Tot. B	Tot. S	Ratio of B: AB	Ratio of S: AB
<i>a</i>	$AB \times AB$	$AB^* \times AB^*$	18	AB(all) . . . . . 16 B & AB . . . . . 2	442 76	442 58	18		1 : 3	
<i>b</i>	$AB \times AB$	$B^* \times B^*$	11	B & AB . . . . . 11	327	57	270		5 : 1	
<i>c</i>	$AB \times S$	$S^* \times S^*$	7	AB & S . . . . . 3 AB, B & S . . . . . 4	181	25 18	8	47 83		2 : 1 10 : 2
<i>d</i>	$AB \times AB$ $S \times S$	$S \times AB$	3	S(all) . . . . . 3	96					
<i>e</i>										

\* Ancestry of each parent is similar.

It is noticeable that the mosaic I noted in the previous generation (Table II) failed to recur.

Table III, category *a*, shows AB again not breeding wholly true, but predominating more extensively than in the previous generation; that is, there is relatively a larger number of wholly AB broods. Its preponderance in mixed broods appears to be not so great, though fewer mixed broods occurred. Here, again, as in the previous generation, we find an absence of the S character in the offspring.

Extracted B, Table III, category *b*, that is B born of AB parents, produces broods with the B type predominating (5 : 1), and in greater proportion than in the previous generation (Table II, category *d*). The reverse might reasonably have been looked for since in the latter case there were but two generations of AB parents, whilst in the former there are three generations of AB parents. It is in line, however, with the normal prepotency of B over AB.

Hybrid S (Table III, category *c*); that is, S from  $S \times AB$ , a cross that had produced only S offspring when mated with a similarly produced individual, produced mixed broods of two kinds; that is, broods of S and B, the S character predominating, or broods of S, B and AB, the S character again predominating, AB taking second rank and B occurring as a minority. It is possible from the known behavior of S and B with relation to each other, that B from hybrid S is but an offshoot from S and therefore must in this connection be considered with relation to S only.

Category *d* shows pure S (pure with reference to AB) again completely dominating in the progeny of  $S \times AB$ .

Individuals of this generation ceased feeding in the laboratory during the latter part of August, 1905, and were placed for hibernation in a dark, cool, well-ventilated shaft connected with the breeding room. They were isolated by broods.

During the first week of March, 1906, the insects began to move about in the cages. They were then brought into the breeding room and feeding began. A large number of S individuals survived the winter, while but twenty-five AB survived.

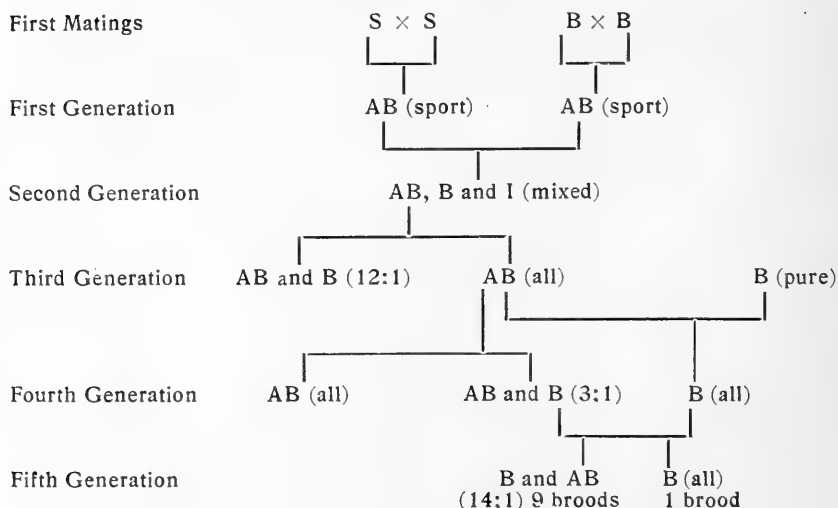
On March 5, 1906, matings were made in the following cate-

gories for a continuation of the study of the hereditary behavior of the AB character.

- Category *a* AB (male or female) of AB parents  $\times$  S of S parents.  
*b* AB of AB parents  $\times$  AB of AB parents.  
*c* AB of AB parents  $\times$  B (collected '06).  
*d* B of B  $\times$  AB parents  $\times$  B of AB  $\times$  AB parents (hybrid B  $\times$  extracted B).  
*e* B of B  $\times$  AB parents  $\times$  B of B  $\times$  AB parents (hybrid B  $\times$  hybrid B).  
*f* S of S  $\times$  AB parents  $\times$  S of S  $\times$  AB parents (hybrid S  $\times$  hybrid S).  
*g* B of AB  $\times$  AB  $\times$  B collected '06 (extracted B  $\times$  pure B (pure with ref. to AB)).  
*h* B of B  $\times$  AB  $\times$  B collected '06 (hybrid B  $\times$  pure B).  
*i* AB ('06 sport)  $\times$  AB ('06 sport).

The following pedigree-diagrams are given for categories *d*, *e*, *f*, *g*, and *h*, in order to show more graphically the character of the lineage.

DIAGRAM VII—Category *d*



In succeeding diagrams the ancestry of at least one parent is similar to that in Diagram VII for the first two generations and hence is not repeated.

DIAGRAM VIII—*Category e*

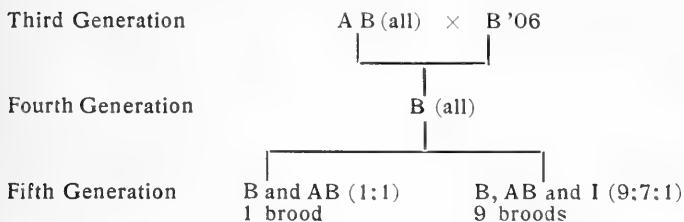


DIAGRAM IX—*Category f*

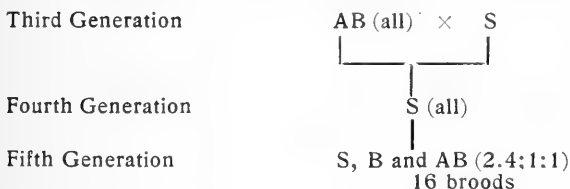


DIAGRAM X—*Category g*

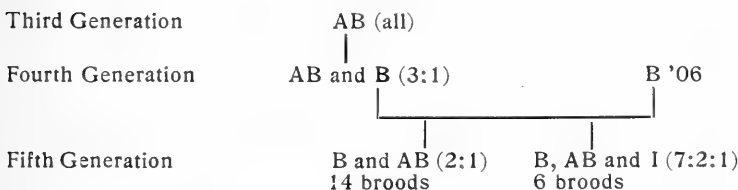
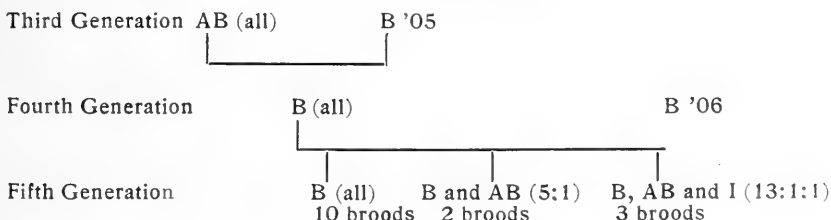


DIAGRAM XI—*Category h*



Behavior of the alternate color characters in this generation is entirely consistent with that in previous generations.

In Table IV, category *a*, S is dominant in every brood (completely dominant in 14 broods, transmitting B in 22 broods). In no case does the allelomorph, AB, appear.

In category *b*, AB is approaching a stage of purity (entirely pure in 27 out of 30 broods). In the three broods in which mosaics occur, there are but four in one brood and two in each of two other broods. Comparison of this category with category *a* of Tables II and III shows here, in the 4th generation from the sport parent an increasing stability of the sport character.

In category *c*, type B is almost completely dominant over the type AB (entirely so, if I is considered to be a modified B in character, as its behavior appears to indicate).

In category *i*, where two '06 sports were mated, the result parallels that of similar matings in the previous year (Table II, category *a*), in the predominance of AB in mixed broods. No broods wholly AB were obtained.

Submitting categories *d*, *e*, *g* and *h* to a closer analysis, we obtain the following data:

Category

*d* Extracted B (from AB×AB) × hybrid B (from AB×B) = 1 B brood, 9 mixed broods (B:AB as 14:1).

*e* Hybrid B × hybrid B = 10 mixed broods  $\begin{cases} \text{B:AB:I as 9:7:1—9 broods.} \\ \text{B:AB as 1:1—1 brood.} \end{cases}$

*g* Extracted B × pure B\* = 20 mixed broods  $\begin{cases} \text{B:AB:I as 7:2:1—6 broods.} \\ \text{B:AB as 2:1—14 broods.} \end{cases}$

*b* Hybrid B × pure B\* = 10 B, 5 mixed broods  $\begin{cases} \text{B:AB:I as 13:1:1—3 broods.} \\ \text{B:AB as 5:1—2 broods.} \end{cases}$

\*Pure in the sense of having no previous contamination with AB.

The following results are noticeable:

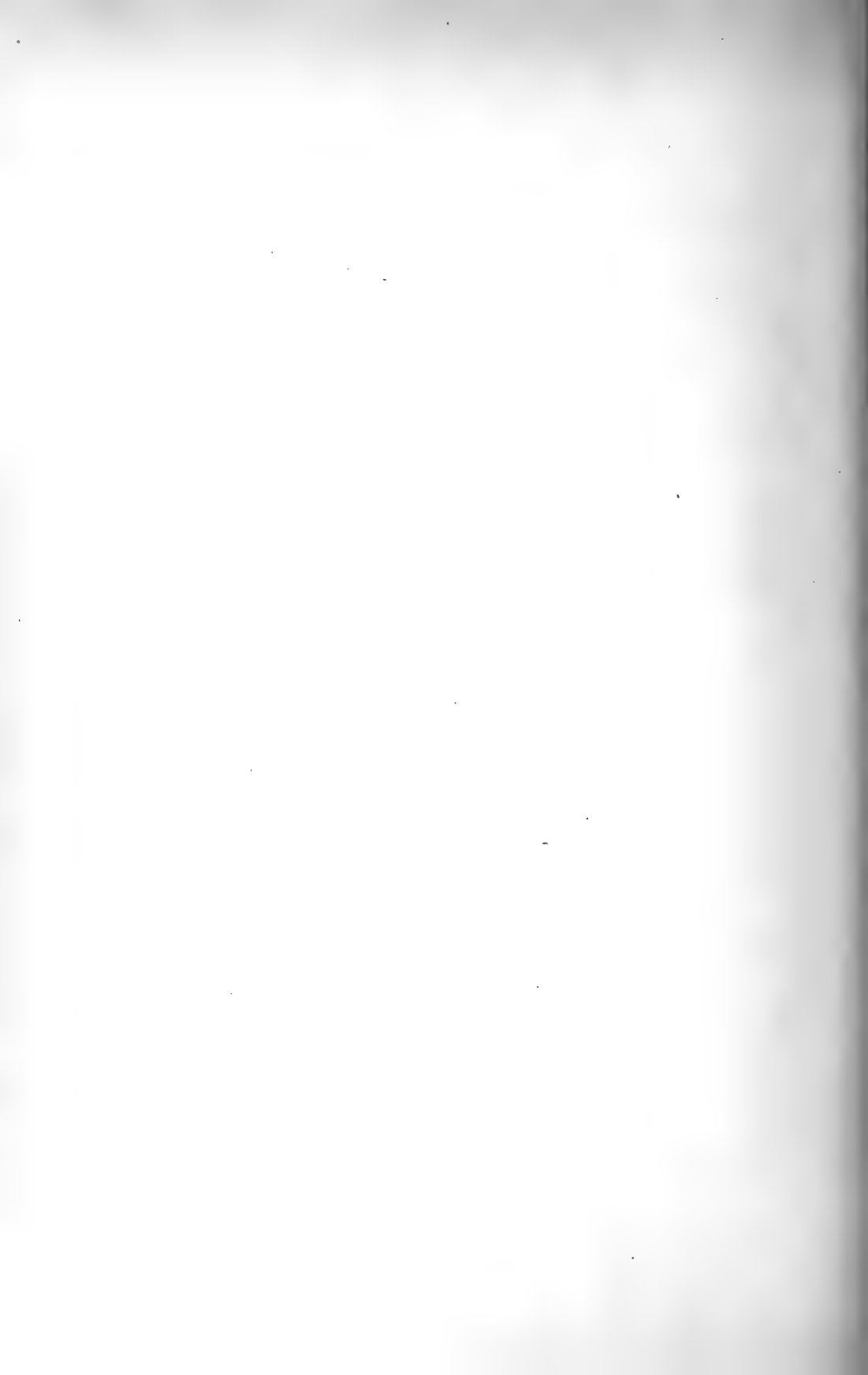
- 1 The total exclusion of S.
- 2 The unexceptional predominance of B over AB and I in mixed broods.
- 3 The large preponderance of exclusively B broods where pure B meets hybrid B.

TABLE IV (Fifth Generation)

Mating category	GGr-Gr. parents	GGreat-Gr. parents	Great Gr. parents	Grandparents	Parents	Total No. Broods	Total No. Individuals	Character of Broods	Proportion of the Respective Classes
<i>a</i>	$\left. \begin{array}{l} S \times S \\ B \times B \\ S \times S \\ B \times B \\ S \times S \\ B \times B \\ S \times S \\ B \times B \end{array} \right\}$	$\left. \begin{array}{l} \text{sport AB} \times \text{sp. AB} \\ " \\ " \\ " \\ " \\ " \\ " \\ " \end{array} \right\}$	$\left. \begin{array}{l} AB \times AB \\ \\ \\ AB \times AB \end{array} \right\}$	AB × AB	AB* × S '06	36	1000	S ..... 14 S and B ... 22	1.14 : 1
<i>b</i>	$\left. \begin{array}{l} " \\ " \end{array} \right\}$	$\left. \begin{array}{l} " \\ " \end{array} \right\}$	$\left. \begin{array}{l} " \\ " \end{array} \right\}$	$\left. \begin{array}{l} " \\ " \end{array} \right\}$	AB* × AB*	30	721	AB ..... 27 AB and I ... 3	7 : 1
<i>c</i>	$\left. \begin{array}{l} " \\ " \end{array} \right\}$	$\left. \begin{array}{l} " \\ " \end{array} \right\}$	$\left. \begin{array}{l} " \\ " \end{array} \right\}$	$\left. \begin{array}{l} " \\ " \end{array} \right\}$	AB* × B '06	22	696	B ..... 10 B and I ... 12	11 : 1
<i>d</i>	$\left. \begin{array}{l} " \\ " \end{array} \right\}$	$\left. \begin{array}{l} " \\ " \end{array} \right\}$	$\left. \begin{array}{l} " \\ " \end{array} \right\}$	$\left. \begin{array}{l} AB \times AB \\ AB \times B '05 \end{array} \right\}$	B × B	10	230	B ..... 1 B and AB 9	14 : 1
<i>e</i>	$\left. \begin{array}{l} " \\ " \end{array} \right\}$	$\left. \begin{array}{l} " \\ " \end{array} \right\}$	$\left. \begin{array}{l} " \\ " \end{array} \right\}$	$\left. \begin{array}{l} AB \times B '05 \\ AB \times B '05 \end{array} \right\}$	B × B	10	311	B ..... 0 B and AB 1 B, AB and I 9	1 : 1 9 : 7 : 1
<i>f</i>	$\left. \begin{array}{l} " \\ " \end{array} \right\}$	$\left. \begin{array}{l} " \\ " \end{array} \right\}$	$\left. \begin{array}{l} " \\ " \end{array} \right\}$	$\left. \begin{array}{l} AB \times S \\ AB \times S \end{array} \right\}$	S × S	16	637	S, B and AB 16	2.4 : 1 : 1
<i>g</i>	$\left. \begin{array}{l} " \\ " \end{array} \right\}$	$\left. \begin{array}{l} " \\ " \end{array} \right\}$	$\left. \begin{array}{l} " \\ " \end{array} \right\}$	AB × AB	B** × B '06	20	369	B and AB 14 B, AB and I 6	2 : 1 7 : 2 : 1
<i>h</i>	$\left. \begin{array}{l} " \\ " \end{array} \right\}$	$\left. \begin{array}{l} " \\ " \end{array} \right\}$	$\left. \begin{array}{l} " \\ " \end{array} \right\}$	AB × B '05	B** × B '06	15	407	B (all) ..... 10 B and AB 2 B, AB and I 3	5 : 1 13 : 1 : 1
<i>i</i>					AB '06 × AB '06	8	216	B and AB 5 B, AB and I 3	1 : 3 1 : 2 : 1

\*The ancestry of the AB parent only is given; in category *a* mated with pure S, in category *b*, mated with AB of similar ancestry, in category *c*, mated with pure B.

\*\*The ancestry of this B parent only is given, the other being a pure B.



4 Extracted B (B from AB×AB) transmits the sport character.

For sixth generation data the following categories of matings were made:

Category *a* S×S, of AB×S parents.

*b* AB×AB, of AB and AB parents.

*c* B×B of AB×AB parents.

*d* B'06×AB—the latter of AB×AB parents.

The following table shows the results of these matings:

TABLE V (*Sixth Generation*)

Mating Category	Grand-parents	Parents	Total No. Broods	Total No. Individuals	Character of Broods	Proportion of the resp. char.
<i>a</i>	AB×S	S×S	12	296	S..... 4 S & B ..... 3 S,B & AB ... 3 S,B & I .... 1 S & I ..... 1	7 : 1
<i>b</i>	AB×AB	AB×AB	11	282	AB ..... 10 AB & I .... 1	2 I ind. in brood of 17
<i>c</i>	AB×AB	B×B	4	92	B & AB .... 3 B, AB & I .. 1	3.3 : 1
<i>d</i>	AB×AB	AB×B'06	1	23	B (all) ..... 1	

Comparing similar categories in Tables IV and V, we find a similarity of results from similar conditions.

1 Hybrid S (Table V, category *a*, and Table IV, *f*) produces either mixed broods with S predominating or broods wholly S.

2 B extracted from AB (from broods indicated in Table IV, category *i*) transmits AB in the succeeding generation (Table V, category *c*) not breeding true as it does when extracted from S.

3 AB becomes practically pure in the sixth generation (fifth generation from the sport). (Table V, category *b*.)

4 Pure B is again completely dominant over AB in a cross between B and AB (Table V, category *d*), as in Table II, *b*.



For the seventh generation data, the following mating categories were established.

- a* AB  $\times$  AB, of AB ancestry for six generations.
- b* S  $\times$  S of dominant S parents (dominant over AB).
- c* B  $\times$  B of incompletely dominant S parents, and S  $\times$  AB grandparents.
- d* B  $\times$  B of dominant B parents (dominant over AB).
- e* AB  $\times$  AB, extracted AB of S parents and S  $\times$  AB grandparents.

TABLE VI (Seventh Generation)

Mating Category	Great Grandparents	Grand-parents	Parents	Total No. Broods	Total No. Individuals	Character Broods
<i>a</i>	AB $\times$ AB	AB $\times$ AB	AB* $\times$ AB*	22	509	AB.....all
<i>b</i>	S $\times$ AB	S $\times$ S	S* $\times$ S*	31	764	S.....29 S with one I—1 S with one B—1
<i>c</i>	S $\times$ AB	S $\times$ S	B* $\times$ B*	6	173	B.....all
<i>d</i>	B $\times$ AB	B $\times$ B	B* $\times$ B*	5	128	B.....all
<i>e</i>	S $\times$ AB	S $\times$ S	AB* $\times$ AB*	5	120	AB.....all

\*The ancestry of each parent is of similar character.

Comparing sixth and seventh generations we find in the latter the alternate B completely eliminated from the AB line (Table V, *b*, and Table VI, *a*).

S in the second generation from hybrid S (Table VI, *b*) breeds true as far as AB is concerned. Its dominance over AB is therefore progressive. There is a much more rapid elimination of AB at this stage than previous breeding experiments have shown to be the case with B.

B in the second generation from hybrid B breeds true (Table VI, *d*). Its dominance over AB is therefore also progressive.

Extracted B from hybrid S breeds true at once (Table VI, *c*).

Extracted AB from hybrid S (Table VI, *e*) breeds true apparently in the first generation.

Unfortunately no matings were made with extracted AB from hybrid B.

#### SUMMARY OF RESULTS

1 That a number of totally black (AB) individuals were collected out-of-doors shows that the type is a natural type.

2 AB, as a sport, mated with its own type, produced, in the first generation, broods in which the sport type predominated; in later generations the alternative character was gradually eliminated.

3 AB as a sport transmits the character B, but fails completely to transmit the character S.

4 S and B completely dominate the type AB.

5 Extracted AB (AB that has become recessive through a cross with S and has later been recovered) produces AB only.

6 Hybrid S (S from S and AB) transmits AB, it may produce broods that are wholly S or mixed broods of S and AB, in which individuals of the S character predominate.

7 Hybrid B (B from B and AB) transmits AB; it produces mixed broods with individuals of the character B predominating.

8 AB is eliminated from the S or B line in the second generation from a cross between S and AB, or B and AB, hence the sport type, although inherently stable, is swamped in back-crossing.

9 Extracted B from hybrid S ( $S \times AB$ ) breeds true.

10 Extracted B from the sport AB ( $AB \times AB$ ) transmits AB, but predominates in the broods.

11 The character I, a mosaic of B and AB, occurs. It has no apparent stability, but may appear in any cross into the lineage of which AB has been introduced.

There is no general adherence to Mendelian proportions in the behavior in inheritance of the sport AB in *Melasoma scripta* but it behaves as a Mendelian recessive in first crosses with both S and B, breeding true from hybrid S.

## CONCLUSION

The characters S, B and AB in this species are mutually exclusive. The conditions of dominance and recessiveness are relative conditions. B is recessive to S, dominant over AB. It is latent in AB as a sport. S is dominant over both B and AB. AB is recessive to both S and B. It may contain latent B, but apparently does not contain latent S. AB as a sport is a stable character, not at first absolutely so, but by selection becoming quite as much so as either S or B.

That there is no apparent tendency toward trichromatism taking the place of dichromatism in the species, seems to be due to the as yet very small proportion of AB individuals normally produced, the remote chance of an AB type meeting with a mate of similar type and the swamping effect of back-crossing.

In this species, therefore, it seems, as suggested by Darwin in 1859,<sup>5</sup> that owing to the "nature of the organism," each type would ultimately become a fixed type if the "nature of the conditions" favored it. The "nature of the organism"—that is, the origin of somatic variations having hereditary value, the relation of these in the germ-cell elements or the inherent quality that makes for dominance or prepotency and the quality that makes for purity—this is the obscure point.

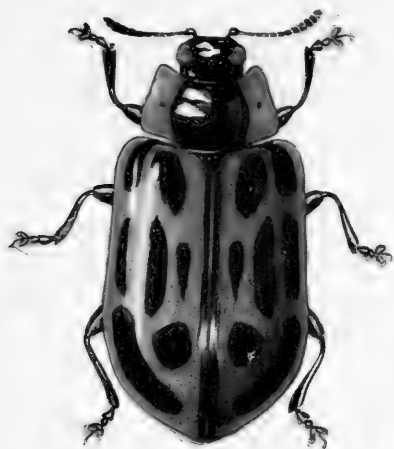
<sup>5</sup>Darwin: Origin of Species, p. 77.

## EXPLANATION OF PLATE

- Fig. 1 *Melasoma scripta* (Fabr.) type with spotted elytra. (S)  
Fig. 2 *Melasoma scripta* (Fabr.) type with black elytra. (B)  
Fig. 3 *Melasoma scripta* (Fabr.) all black sport of occasional occurrence. (AB)  
Fig. 4. *Melasoma scripta* (Fabr.), mosaic type of 2 and 3 resulting from intermating or back crossing 3.

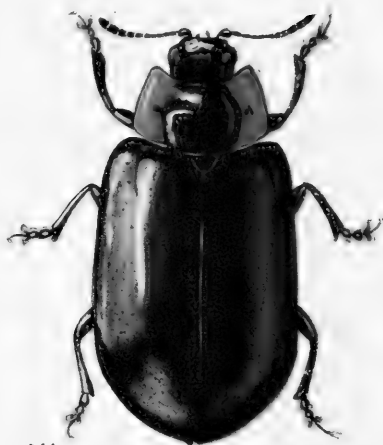
SPORT IN MELASOMA AND ITS BEHAVIOR IN HEREDITY

ISABEL McCracken



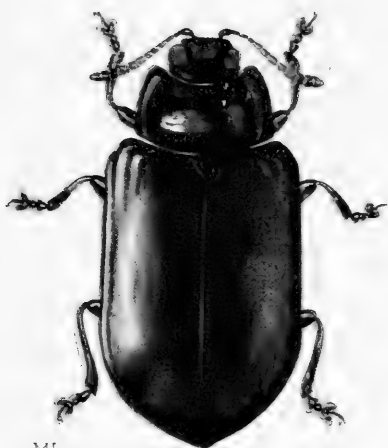
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*Fig. 1*



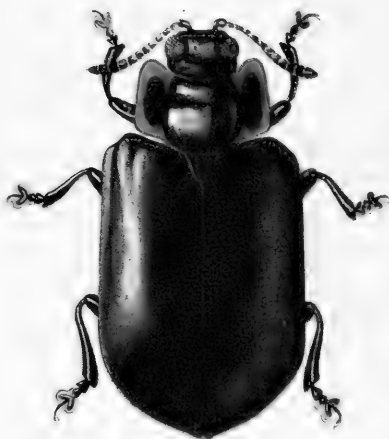
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*Fig. 2*



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*Fig. 3*



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*Fig. 4*



# EXPERIMENTS IN TRANSPLANTING LIMBS AND THEIR BEARING UPON THE PROBLEMS OF THE DEVELOPMENT OF NERVES<sup>1</sup>

BY

ROSS GRANVILLE HARRISON

WITH FOURTEEN FIGURES

Several years ago Braus described a series of ingenious experiments in transplanting limbs of amphibian (Bombinator) larvæ. The experiments were made mainly for the purpose of inquiring into questions relative to the development of peripheral nerves and their author has interpreted his results in accordance with Hensen's theory. Briefly stated this theory is that the nerve centers and their peripheral end-organs are connected from the beginning of embryonic life by means of protoplasmic bridges, and that the development of the nerve fiber consists merely in the differentiation of these pre-existing connections under the stimulus of functional activity. Banchi, whose results have, however, been contradicted by Gemelli, has also made transplantation experiments and like Braus has been led to the conclusion that the peripheral nerves develop *in situ* and that they may undergo their development even when not connected with ganglion cells. My own experiments lend, on the other hand, no support whatever to the Hensen theory but show in agreement with His that the nerve fiber is the outgrowth of the ganglion cell.<sup>2</sup> It is this difference of opinion that has led to the following study, which is

<sup>1</sup>A brief account of the experiments described below was given in a paper read before the Section in Anatomy of the British Medical Association at the meeting held in Toronto, August 21-25, 1906. The work was reported more fully to the Association of American Anatomists at the New York Meeting, December 29, 1906. See Anatomical Record, Am. Journ. Anat., vol. vi, no. 3.

<sup>2</sup>Harrison '04a, '06.

based upon a new series of experiments similar to those of Braus and Banchi. Before describing the new experiments it will be necessary to consider the previous work in detail, beginning with that of Braus, the main facts of which are as follows:

1 If an extremity is taken at a time when it is beginning to develop and is transplanted to any region of another tadpole, it will continue its development in the new position and ultimately be found to contain all of the parts—muscles, skeleton, blood vessels and nerves—which are perfectly normal both as to structure and arrangement. The nerves are connected with the nerves supplying the region of the host into which the limb has been implanted, as, for instance, with the facial nerve when the limb is placed on the side of the head in the region of the orbito-hyoid muscle, or with the nerves of the lumbo-sacral plexus when a fore limb is transplanted to the hind limb region. But the nerves within these transplanted extremities, in spite of their unusual origin, ramify exactly as the nerves in a limb in its natural position. Furthermore, these nerves are functional, both voluntary movements of the limbs and movements in response to electrical stimulation having been observed. In considering this experiment, Braus lays great stress upon the point that at the time of transplantation either there are no visibly differentiated nerves within the limb,<sup>3</sup> or, if nerves are present, they degenerate and disappear very soon after; the latter was the case in his earlier experiments.

2 In a second series of experiments limb buds were taken from larvæ, from which the whole spinal cord had been removed at a period just after the closure of the medullary folds. Such embryos develop normally aside from the defect caused by the wound, but they contain no nerves except those arising in the head.<sup>4</sup> Extremities taken from individuals of this character Braus terms “aneurogenic,” in contradistinction to the normal ones which are called “euneurogenic.” The aneurogenic buds were taken from the nerveless larvæ ten days after the removal of the spinal cord and implanted into the hind limb region of normal individuals. Eight days later these larvæ were preserved but, although the transplanted limbs had developed considerably, no nerves were found in them, whereas a normal or euneurogenic transplanted bud would have contained nerves by this time.

3 The normal transplanted limbs acquire, as stated above, nerves which take a normal course. Examination of such limbs in a state of incomplete development, for example, about three weeks after transplantation, shows that the nerve trunks within them are much thicker than the nerves of the host with which they are connected.

<sup>3</sup>Braus '05, p. 438.

<sup>4</sup>Harrison '04.

4. It happens not infrequently that the transplanted extremity does not remain single, but gives rise to a second limb which is the mirror image of the original one. This accessory limb is at first small but after a time may overtake the original one in development. Such supernumerary limbs may also be formed after irregular amputations or mutilation of the limb rudiment in normal tadpoles,<sup>5</sup> in which case the nerves as well as the other structures are typically developed. On the other hand, when the supernumerary limb develops from a transplanted bud, Braus finds that nerves are totally lacking within it, as indicated both histologically and by the failure of such limbs to respond to electrical stimulation.

Aside from these main points Braus' papers contain a wealth of interesting and important observations. His conclusions are however, interwoven with his statements of fact and are presented in the form of a continuous argument, thus rendering it difficult to bring effective criticism to bear upon the work. Nevertheless, by first admitting the facts to be true, we may take up the thread of the argument and, I think, conclusively show that it does not constitute a logical proof of the continuity theory, but is merely to be regarded as a possible interpretation, in which analogies and collateral facts of uncertain relevance play a large rôle. The same facts may be interpreted quite as readily, if not more so, in accordance with the outgrowth theory. The experiments do not approach the problem directly enough to determine questions of histogenesis, and there are too many loopholes left to permit of a rigid proof. In the experimental part of the present paper it will be further shown that the facts which are most important for Braus' argument are not of general validity.

In considering the experiments just referred to under the first heading, Braus attacks the question as to the origin of the nerves in the transplanted limbs.<sup>6</sup> Are the nerves developed out of struc-

<sup>5</sup>Barfurth '94; Tornier '05.

<sup>6</sup>Braus states this problem in somewhat different form: "Um dies zu erläutern, möchte ich zunächst die Frage zu beantworten versuchen: an welcher Stelle verbindet sich bei meinen Kompositionen die motorische Bahn des Autositen mit derjenigen des Parasiten?"

\* \* \* Es müsste deshalb auf der ganzen Ausdehnung der motorischen Bahn nach der Verwachsungsstelle gesucht werden; doch kommen naturgemäss auf dieser 3 stellen hauptsächlich in Betracht: (1) die Stelle, an welcher die peripheren Nerven mit den Muskeln der transplantierten Gliedmasse in Verbindung stehen, d. h. also innerhalb des Parasiten; (2) die Stelle, an welcher die Einpfropfung erfolgte und wo primär bei der Operation die Gewebe der beiden verwendeten Embryonen



tures already present in the limb at the time of transplantation, or do they grow into the limb from the nerves of the host, guided to their proper place by the structures within the limb? Obviously the experiment under consideration is not, in itself, sufficient to dispose of the question, though Braus contends, that when we clearly analyze the meaning of the one central fact, that the transplanted limb contains a typically arranged nervous system, the first of the three possibilities stated by him, *i. e.*, the formation of the nerves by outgrowth from the center, is to be excluded.

The results of nerve suturing are dismissed as having no bearing on the case, on the ground that there are no nerves present in the limb at the time of transplantation to serve as guide-lines for possible outgrowing nerves. In making this point, however, Braus has failed to note that in normal embryos the nerves reach the immediate vicinity of both the fore and hind limb buds before these appendages are distinguishable.<sup>7</sup> It would, therefore, be impossible to remove a limb bud—and this is especially true of the fore limb (Fig. 1), which Braus used in the majority of his experiments—without including the finer terminal nerve twigs. It would also be impossible to make the incision for implantation without cutting some nerves of the host. Thus in grafting a normal limb upon a normal larva we cannot avoid bringing the cut ends of the nerves of the latter into close proximity to the isolated nerve twigs contained within the former. The two are not actually sutured together, but as Forssmann has shown conclusively, degenerating nervous tissue attracts from a distance the nerve fibers arising from a cut nerve,<sup>8</sup> and the distances in the embryonic transplantations under consideration are sufficiently minute to fall well within this limit. We must therefore consider that also in these embryonic transplantations guiding nerve twigs may be present, though even were they totally lacking in the transplanted bud, the nerves of the host would be brought into close

aneinander geheilt wurden, d. h. also innerhalb der Vereinigungsstelle des Parasiten und Autositen; (3) die Stelle, an welcher das periphere Nervensystem mit dem zentralen, speziell den motorischen Ganglienzellen des letzteren zusammenhängt, d. h. also innerhalb des Autositen." Braus '05, p. 442.

<sup>7</sup>See p. 253.

<sup>8</sup>Forssmann '98, '00.

proximity with the latter, while it is still nothing but a small nodule of mesenchyme cells, and there is no reason to suppose that these nerves would have any more difficulty in making proper connections, than the nerves have in growing into the natural limbs. It would therefore seem to be almost immaterial from the standpoint of the outgrowth theory whether nerve twigs are present in the limb at the time of transplantation or not, and my own experiments show that this is actually the case.<sup>9</sup>

Braus nevertheless finds an insuperable objection to the acceptance of the outgrowth theory in the fact that the grafted limb is entirely new territory for the nerve, where we should not be justified in supposing the latter to be able to find its way. But this difficulty would be real, only in case it could be shown that the nerve fibers going to each organ are specific, *i. e.*, that the mode of branching of the fibers is determined in the nerves themselves. While it may be true that there are some specific differences between, for example, muscular and cutaneous nerve fibers, there is no evidence to show that the nerves running to any particular muscle are different from those supplying other muscles. In fact our experience with nerve anastomosis shows that the contrary is the case. There is no difficulty, therefore, in the supposition that, if the structures in the fore limb normally direct the course of the nerves of the brachial plexus, they may direct equally well the lumbo-sacral nerves, when, as in the case of the transplantations, the fore limb is placed in the way of the latter.

Braus supports his contention mainly upon the supposed analogy with the behavior of the rudiment of the lateral line organs in development, but in so doing he has partially mistaken the meaning of my experiments which he cites.<sup>10</sup> While these experiments did show that the rudiment of the sense organs—the nerve itself is merely drawn along with them—does not grow except in normal paths or paths similar to the normal ones, they did not prove that the vagus organs, for instance, could not grow into the supra-orbital path, as would have to be the case were the analogy with the strange nerves in a transplanted limb to hold. In

<sup>9</sup>See pp. 256 and 269.

<sup>10</sup>Harrison '03.

fact my experiments did actually show<sup>11</sup> that the main chain of organs would grow into the path of the dorsal branch when the former was shifted from its normal course, just as we may suppose the nerves of the lumbo-sacral region to grow under the guidance of the paths in the forelimb. Thus the analogy favors the possibility of outgrowth into strange paths and not the opposite as Braus supposes.

We now come to the second main point made by Braus, which is that an "aneurogenic" extremity when transplanted to a normal individual does not acquire a nervous system. Were the nerves formed as outgrowths from the nerves of the host, Braus argues that they should grow just as readily into "aneurogenic" extremities as into the normal ones.<sup>12</sup> Their failure to do so he regards as sufficient ground for rejecting the outgrowth theory.<sup>13</sup> It is true one may interpret this result in accordance with Hensen's theory, though in so doing it must be admitted that the differentiation of the nerve fibers is dependent upon the integrity of their connection with the centers, for the only reason to be given that the nerves have not been formed in the aneurogenic buds at the time of transplantation is because their connection with the centers have been destroyed; not having been in connection during the critical period, their development at any future period is precluded and such limbs remain therefore without nerves. But it is not necessary to refer differences between the "aneurogenic" and the "euneurogenic" extremities to invisible pre-nervous structures for, as already mentioned, there are actual visible nerve fibers present in the normal or "euneurogenic" buds at the time of transplantation, while, of course, in the "aneurogenic" ones there are none.<sup>14</sup> But even this tangible difference between the two sorts of limb buds does not actually cause the nerves to behave differently toward them, unless, possibly, the absence of the nerve twigs in the one case retards to a slight extent the development of the nerves, for, as my

<sup>11</sup>Op. cit., p. 127.

<sup>12</sup>Braus '05, p. 450.

<sup>13</sup>Braus '05, p. 452.

<sup>14</sup>This was given as a possible explanation in my brief criticism of Braus' work (Harrison '06). At that time I did not have the data afforded by my own experiments, which show that as a matter of fact no explanation is necessary.

own experiments show, contrary to those of Braus, a normally arranged nervous system does usually develop in the "aneurogenic" extremities.

The third point which Braus makes is, that while the nerves which connect transplanted limbs with the central nervous system of the host are extremely minute, those within the limb are almost as thick as the nerves in the normal limb. As Braus points out, there are obviously two possible arrangements which may account for this condition; either there are some fibers in the transplanted limb that are not connected with nerve fibers of the host, or the fibers connecting with the host branch frequently as they enter the grafted appendage. Since it has not been possible to determine by direct observation which of these alternatives is true, Braus has attempted to settle the question indirectly. He is led to reject the latter alternative and in accepting the former concludes that the fibers in the grafted leg are developed autochthonously. The first consideration which Braus adduces against the idea of division is that the number of neuro-fibrils in the peripheral part of the nerve is much greater than in the connecting strands, and since according to Apáthy the fibrils in peripheral nerve fibers do not bifurcate, the peripheral increase cannot possibly be accounted for in this way. This, to say the least, is an extremely hazardous conclusion to draw, for it has never been shown with any degree of certainty that the fibrils do not divide in the axones of vertebrates. In fact there is now considerable positive evidence that branching does take place. Dogiel has shown this to be the case in the nerves running to the corpuscles of Herbst and Grandry, and Ramon y Cajal in certain cortical axones. Retzius has demonstrated that the fibrillæ increase in number on either side of the nodes of Ranvier and quite recently Schiefferdecker has confirmed and extended this observation, showing that in peripheral nerves of the frog finer fibrils coalesce at the nodes of Ranvier into a small number of much coarser threads, which, after the node is passed again break up into the finer fibrillæ. In other words the neurofibrils of a peripheral nerve form a network.<sup>15</sup>

<sup>15</sup>Schiefferdecker ('06a) discusses at length the relations and mode of branching of the fibrillæ both in the cell body and in the axis cylinder.

In view of these facts the observation of an increase in the number of fibrillæ in a given peripheral nerve as its end is approached can have no weight in support of the hypothesis that some of them are lacking connection with the center.<sup>16</sup>

There is another consideration which Braus takes up in support of his explanation of the greater thickness of the nerves within the transplanted extremity. He says, if one follows the development of the specimens until the nerves become myelinated, one finds on counting the fibers that there are now no more within the transplanted limb than in the connecting nerves. This shows, it is maintained, that, of the numerous fibers in the transplanted limbs after metamorphosis, when its functional activity comes into consideration, only those which are connected with fibers in the host get myelin sheaths; the superfluous fibrillæ supposedly disintegrate. It would require study of a large number of instances to prove that this change in the numerical relation between the intrinsic and extrinsic nerve fibers of the limb during the course of development is of general occurrence; yet the establishment of this fact would give us only one of the necessary premises requisite to put Braus' conclusion upon a logical basis. Braus does not mention the exact number of cases studied, but actual figures are given in only a single instance. Now the relation of the size of the nerve trunks within the limb to that of the connecting nerves of the host, is very variable, as my own cases show, and hence it is quite within the bounds of possibility, not to speak of proba-

<sup>16</sup>Curiously enough Braus calls to the support of his view the conditions found in the nerve to the electric organ of *Malapterurus*, where, as is well known, the organ of each side is innervated by a single large cell. Since the single fiber derived from this cell ultimately breaks up into several million twigs, one would naturally suppose that this would show pretty conclusively that the fibrils must branch. As a matter of fact it does, but Braus in maintaining the contrary is misled by the fact that the main nerve is described as of very large caliber. The actual figures given by Fritsch show at once the untenability of Braus' position. While the nerve is as a matter of fact enormous, having a diameter of 1, 1 mm., its thickness is accounted for by its sheaths. The axis cylinder is but 8  $\mu$  in diameter. There are estimated to be 2,171,252 electric discs in the organ, each of which receives a nerve, the combined area of cross section of the terminal stalks being 346,760 times the area of cross section of the original fiber. Assuming each terminal twig to contain but a single fibril, though probably there are more, and that there is no branching, the main axis cylinder would have to contain at least 2,171,252 fibrillæ, which is of course an absurdity, for in order that so many be squeezed into such small compass the individual fibril, even allowing no space at all for interfibrillar substance, could not be more than about .005  $\mu$  thick, which is far below the range of visibility.

bility, that in the case which Braus cites the relative size of the different parts of the nerve trunk was the same in the earlier stages of development as when finally enumerated. I have not found in any of my experiments such marked differences in size between the intrinsic and extrinsic portions of the nerves as Braus found in the case figured;<sup>17</sup> and there may even be no enlargement at all. Furthermore, sudden thickening of the nerve trunks do not take place exclusively at the point where they enter the limb but may take place at any dividing point within the limb.

In this connection it is interesting to note that the exact enumeration of the nerve fibers in the hind legs of adult frogs, made by Dunn, shows that the number of medullated fibers increases as the periphery is approached.<sup>18</sup> This increase is not confined to the finer nerve twigs but begins to manifest itself in the sciatic nerve where Dunn finds that the increase is from 6 to 8 per cent. This is due to bifurcation of fibers; branching fibers of large caliber were actually observed in teased preparations. When, therefore, we explain the peripheral enlargement of the nerves as they enter the transplanted extremities as due to division of the axones and not, as Braus holds, to the existence of fibers having no connection with the center, we are calling into consideration a condition which differs from the normal merely in degree, not in kind. This view is, in other words, in accordance with known facts relating to the peripheral distribution of nerves, while the explanation given by Braus can at best be regarded as an uncertain hypothesis.

The fourth point which Braus makes is of somewhat the same nature as the second, and is considered by him as showing that the origin of the nerve fibers is to be sought in a much earlier stage of development than that in which either the cell outgrowth theory or the cell chain theory of Balfour place it.<sup>19</sup> It is maintained that no nerves are formed in the accessory limbs which often arise from the transplanted buds, and that this is a distinct confirmation of the argument against the outgrowth theory, because this

<sup>17</sup>Braus '05, Fig. 12A, p. 454.

<sup>18</sup>This was pointed out by Professor Donaldson in the discussion which followed the reading of my communication before the British Medical Association.

<sup>19</sup>Braus '05, p. 460.

theory is unable to explain why the nerves do not grow into the accessory as well as into the primary limb. In reply to this, it may be pointed out that while it might be difficult to explain why the nerves never *grow* into such limbs, there is nevertheless a very natural explanation, not considered by Braus, why they might often be easily overlooked. This explanation is based upon the fact given by Braus that the nerve trunks in transplanted limbs contain only a small fraction of the fibers found in a normal limb. When the secondary limb buds out, it derives its nerves, therefore, from a very much attenuated nervous system, and of course it receives only a very small fraction of the fibers making up the latter. Supposing, for example, that the transplanted bud receives but one-tenth of the normal number of fibers and the accessory bud but one-tenth of these, then the latter would receive but one hundredth part of the normal number, which would render the nerves so minute that they might escape observation. This actually happened in one of my cases when the sections were gone over the first time, though afterward the much attenuated nerves were observed and found to be fairly complete. In other cases, both in accessory limbs derived from "aneurogenic" buds and in those from normal buds, the nervous system was well developed. These facts show that while Braus' statement regarding the absence of nerves from the accessory limbs may hold true in particular cases, as a generalization it is incorrect.

In reviewing the above considerations, it may be fairly said that there are no facts brought out in Braus' contributions which cannot be interpreted in an unforced way in accordance with the view that the nerve fiber is the outgrowth of the ganglion cell. It is not contended that the facts *prove* the truth of this conception, but merely that they do not antagonize it. When, on the other hand, they are interpreted in the light of Hensen's theory, Braus finds it necessary to make a number of subsidiary assumptions which are not always consistent with one another. I may refer especially to the position which is taken regarding the formative influence of the nerve center upon the developing fiber. Thus in explaining the difference between the "aneurogenic" and "euneurogenic" limb buds, Braus supposes that a connection with the center is necessary in the

early stages of development in order that the nerve fibers may afterward differentiate; then to explain the greater thickness of the intrinsic nerves of the transplanted limb in comparison with the connecting nerves within the body of the host, it is assumed that nerve fibers may develop autochthonously within the limb without having any connection with the nerve centers; finally to account for the reduction of the number of fibers within the transplanted limbs after myelinization sets and the muscles become functional, the connection with the center is again supposed to be necessary. A change of this kind in the necessary conditions governing the well-being of the nerve fiber is, on its face, extremely improbable and any theory requiring such inconsistent assumptions for its support, as the Hensen theory seems to require in this case, must be regarded as altogether unsatisfactory.

We may now pass to the consideration of the work of Banchi, who claims to have found within transplanted limbs nerves which had no connection whatever with the nerves of the host. Banchi's method of procedure is somewhat different from that of Braus, one difference being that younger embryos were used for the experiments. It is, however, an error to maintain that the pieces transplanted contain no traces of vessels or nerves,<sup>20</sup> for spinal nerves reach at the stage in question the primary abdominal muscle, which skirts the hind limb rudiment and which Banchi shows in his figure. These nerves may be readily observed in well stained sections, though it is necessary to use high powers of magnification to bring them out clearly. They may also be readily demonstrated in preparations of the abdominal walls *in toto*. What Banchi has gained over Braus by operating on earlier stages he has lost in using such extensive pieces. It is probable that the pieces which the former transplants contain more nerve fibers and Schwann cells than those of which Braus has made use of. When we examine Banchi's experiments we find that out of twenty-nine cases which are described there are only two in which the transplanted limbs contain nerves purporting to have no connection

<sup>20</sup>This is maintained in one place though in the conclusion the statement is considerably modified. Cf. Banchi '05, pp. 675 and 689.



with the nerves of the host. In three other cases the nerves which were found were connected with the nerves of the host. With reference to the other twenty-four the author does not mention nerves except in two cases, in which, it is stated, the relations of the nerves could not be made out.

In a later paper Banchi gives an account of some further experiments, in which specimens were preserved for examination at various intervals after the operation, beginning at six days. The pieces transplanted were not limb buds but pieces of the side of the body, including parts of myotomes and notochord and also, as Banchi admits, portions of spinal nerves. Examined later, it is maintained that in some cases well developed nerves may be found which contain no traces of ganglion cells and have no connection with the nerves of the host. Banchi concludes from these experiments that the nerves are of pluricellular origin and that they may differentiate when cut off from the center. Banchi then points out that what he has found in the field of development corresponds to what Bethe has found in the field of regeneration.

These last experiments cannot be regarded as any more conclusive than the first. It must not be lost sight of that Banchi has really transplanted nerves already partially differentiated; at best he has shown that these nerves are able to continue their existence and perhaps grow a little after being cut off from their ganglion cells. But the experiments do not even prove this, for the pieces were transplanted to other embryos which are teeming with nerves, and in such cases there is always the possibility, or even the probability that connections with the nerves of the host were present but overlooked. The small number of cases purporting to give positive results lends strength to this view. As Braus has shown, the connecting nerves may be very much thinner than the nerves in the grafted piece itself, and it is not difficult to overlook nerves of considerable size, unless one carefully examines the series of sections with an oil immersion lens.

Gemelli's results contradict Banchi's directly and are of special importance because some of the actual steps by which the ingrowth of the nerves into the transplanted tissue were observed. Specimens were killed at short intervals beginning the second day after

operation. After the fourth day Gemelli was in all cases able to detect nerve filaments which arose from the nerves of the host and extended toward the grafted appendage; in somewhat later stages it was found that these nerves entered the latter and that they in all cases preserved their connection with the nerves of the host.

When, therefore, we consider that Braus, Gemelli and myself have never found cases in which the nerves of the transplanted limb were not connected with the nerves of the host, and also that Banchi himself claims to have found only a small proportion of cases in which no connection could be traced; and further, when we consider that a ready source of contamination was present, and that small nerves in the embryonic body may easily elude observation, we cannot but conclude that Banchi is in error in the interpretation of his material, and that there is as yet no satisfactory proof for the claim that nerves may differentiate in a peripheral part when nervous connection with the center does not exist.<sup>21</sup> In order to prove definitely this important proposition, it is necessary that every possible precaution be taken to prevent the formation of anastomoses. The experiments upon which the present claims in favor of the autogenetic development of nerve fibers are based do not measure up to this standard, and the position of those who are now advocating this view on the strength of these experiments, in which sources of contamination were not eliminated, presents a strong resemblance to the position formerly taken by the advocates of abiogenesis, while in fact there is no more evidence for the one than for the other.

#### EXPERIMENTS

The experiments, consisting of two series, which are to be described below, were made upon larvæ of *Rana sylvatica* and *Bufo lentiginosus*.

In the first series limb buds taken from nerveless or "aneurogenic" larvæ were transplanted to normal individuals. The nerveless specimens were produced, as described previously,<sup>22</sup> by

<sup>21</sup>The question of autoregeneration of fully differentiated nerves is here left out of consideration, the writer reserving his opinion on this matter.

<sup>22</sup>Harrison '04, p. 201.

cutting off from the dorsal edge of the body of the embryo at a stage when the medullary folds had just closed, a thin strip containing the spinal cord. At the time of this operation no visible differentiation of fibers had taken place either within the central nervous system or without, and the specimens passed through their further development in the entire absence of peripheral nerves except those derived from the head region, which had not been injured. They lived and developed normally until the yolk absorp-

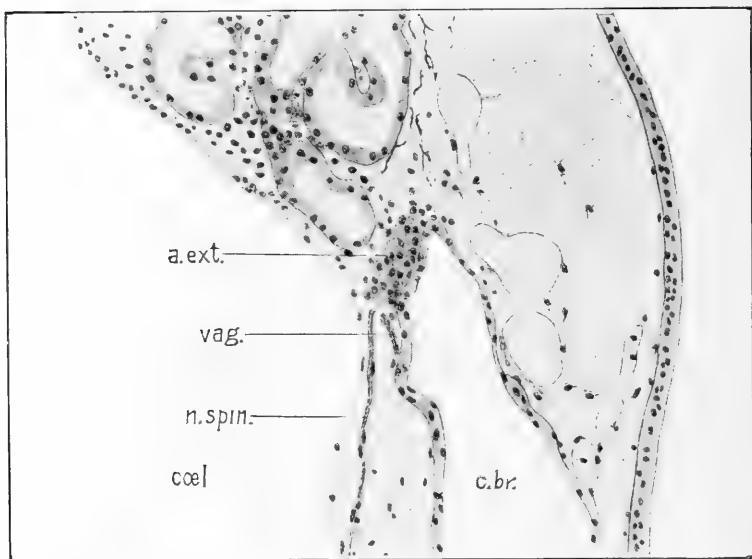


Fig. 1 Cross section through the pronephric region of an embryo of *Rana palustris* 12 mm. long, in which the absorption of the yolk is almost complete. *a. ext.*, fore limb; *vag.*, abdominal branch of the *r. lateralis vagi*; *n. spin.*, ramus abdominalis *n. spinalis*; *cœl.*, body cavity; *c. br.*, branchial cavity.  $\times 133$ .

tion was complete or nearly so, a period varying with the temperature from seven to nine days.<sup>23</sup> At the end of this time the hind limb buds were transplanted to normal larvæ of the same age.

<sup>23</sup>Braus transplanted the nerveless limbs ten days after the removal of the spinal cord. The shorter period of time represented in my experiments does not mean that the embryos had developed less than those used by Braus but simply that they had probably developed at a higher temperature. It was deemed unwise to keep them longer, for degenerative changes are very rapid after the yolk is absorbed, and death soon follows.

These experiments were controlled by transplanting normal hind-limbs, to the same individuals that had received the nerveless buds, the latter having been placed on the right side of the trunk, the former on the left.

At this period of development the rudiments of both the fore and hind limbs are little knobs or buds which project slightly from the surface of the body, the fore limb being concealed, however, by the operculum. Each of the limbs consists of a group of mesenchyme cells situated in the body wall between the somatopleuric

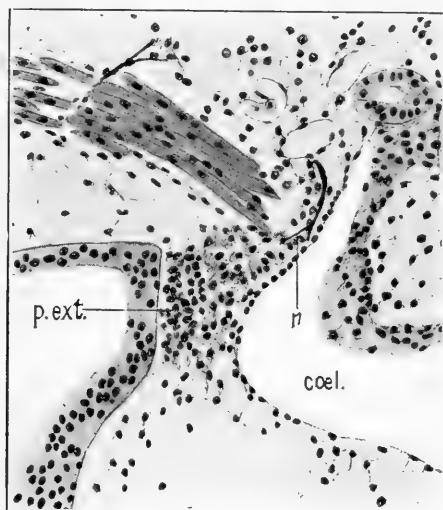


Fig. 2 Sagittal section through the hind limb region of a larva of *Rana palustris* 11 mm. long slightly younger than the embryo from which Fig. 1 was taken. *p. ext.*, hind limb; *coel.*, coelom; *n*, nerve reaching nearly to base of limb.  $\times 133$ .

mesoderm and the epidermis. The cells at this period are closely crowded (Figs. 1 and 2), but as yet no differentiation of tissues is noticeable. There is no visible difference between the limbs in the normal and nerveless individuals except in respect to the nerve fibers, which either run to or skirt past the base of the normal ones. In this respect my own observations cannot be brought into accord with those of Braus, who denies the presence of nerves in the normal limbs at this time. A careful study of numerous series of sec-

tions of *Rana* embryos in closely graded developmental stages will convince one that nerves are present in the immediate vicinity of both the fore and hind limbs as soon as the limbs themselves become distinguishable, and in the case of the fore limb (Fig. 1), even before any signs of the mesenchymatic thickening can be made out.

The specimens were kept alive for a considerable length of time, in most cases until the hind limbs, both natural and transplanted, were fairly well developed and showed the various articulations. In this respect my own experiments differed from those of Braus, who kept none of his "aneurogenic" transplantations alive longer than eight days. It is quite probable that this difference would be sufficient to account for the divergence of our results.

In the second series of experiments, which were made to test the power of autochthonous development of peripheral nerves, normal hind limb buds were transplanted to nerveless organisms. Braus recognized the importance of this experiment and attempted to carry it out, though without results, for the reason that the nerveless tadpoles were unable to maintain themselves alive for a sufficient length of time. This difficulty was obviated in the present case by providing each nerveless individual with a nurse, *i. e.*, by uniting it to a normal larva, in the fashion of Siamese twins (Fig. 14). The experiments were only partially successful, however, because for some unknown reason none of the transplanted limbs grew well on the bodies of these nerveless larvæ.

#### *Transplantation of Normal and Nerveless Limbs to Normal Individuals*

The four experiments to be considered under this heading will be taken up individually.

*Experiment I.*<sup>24</sup> The three larvæ (*R. sylvatica*) used for this experiment were reared together under the same external conditions. On April 15, 1906, shortly after the medullary folds had closed over, the medullary cord behind the vagus region was entirely removed from one specimen. On April 22, the absorption of the

<sup>24</sup>Record number, Tr. Ext. 7.

yolk being nearly complete, the transplantation of the limbs was undertaken. During the intervening period the normal specimens had developed a little more rapidly than the nerveless one, the external form of which is shown in Fig. 3. The limb buds were transplanted to the side of the body of one of the normal individuals, dorsal and anterior to its natural hind limbs, the right limb from the nerveless individual being placed on the right and the left limb of the normal one on the left.

After removal of the hind limb for the purpose of transplantation the nerveless larva was preserved and afterward cut into sections. It was found that the operation for the removal of the spinal cord had been entirely successful. The central nervous system ends abruptly immediately behind the auditory vesicle and no ganglion cells are to be found posterior to the vagus. The brain has undergone marked histolytic changes, practically no



Fig. 3 Embryo from which the nerveless limb in Experiment I was taken.  $\times 9$ .

normal nerve cells being left in it. No longitudinal bundle fibers have grown out posteriorly from the brain and there are no signs of regeneration. The only nerves to be found in the body are those arising in the head. It is therefore certain that the limb transplanted from this individual was entirely nerveless.

The tadpole was kept alive until May 22, during which time it grew rapidly and both of the transplanted limbs as well as the normal ones developed well. Each one of the transplanted buds gave rise to a pair of legs, as Braus described in some of his cases. The photograph (Fig. 4), which was taken after the specimen was preserved, shows the "aneurogenic" or "nerveless" extremities in addition to the natural ones. The thigh which projects dorsally is part of the primary limb, a right hind leg, while the other leg, a left, which projects directly caudalward, is the accessory one.<sup>23</sup>

<sup>23</sup>Tornier and Braus have shown that the accessory limb is the mirror image of the primary one from which it is budded.

The larva was closely observed while alive, and it was noted that slight movements took place in the "aneurogenic" limb. No attempt was made to stimulate electrically, but the spontaneous movements, though very slight, were unmistakable.

After preservation in Zenker's fluid the specimen was cut into serial transverse sections, and the internal anatomy of the extremities carefully studied. A description of their innervation follows:<sup>26</sup>

The "aneurogenic" limbs are innervated from three segmental

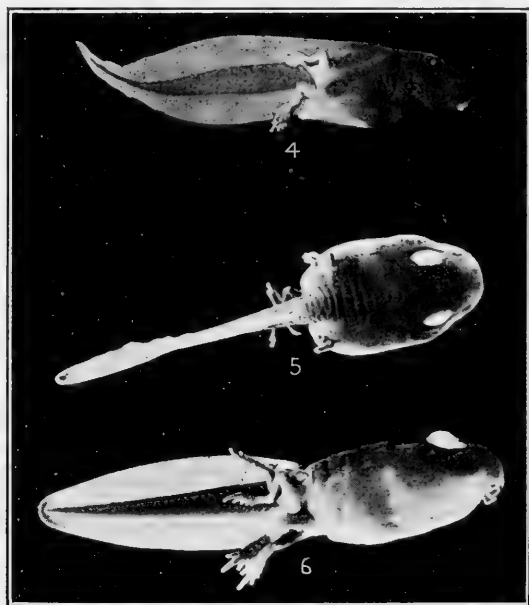


Fig. 4 Subject of Experiment I, showing the "aneurogenic" limbs.  $\times 2\frac{1}{2}$ .

Fig. 5 Subject of Experiment III.  $\times 3$

Fig. 6 Subject of Experiment II.  $\times 3$ .

nerves, the sixth, seventh and eighth. The branch from the sixth is small and does not form anastomoses which are traceable in sections with the others. It runs in the body wall for a considerable distance ventral to the attachment of the limbs, and finally

<sup>26</sup>In this I have been aided by the excellent descriptions given by Gaupp, whose terminology has been followed throughout.

arches backward again running into the primary limb as its r. cutaneus femoris lateralis, a nerve normally derived from the cruralis, which in turn is usually composed of fibers from the eighth and sometimes also the ninth spinal nerves.

The seventh and eighth nerves are large trunks which anastomose freely with one another and it is from this plexus that both the primary and secondary limbs are principally innervated. The arrangement of the plexus is shown in Fig. 7, which was drawn from a graphic reconstruction.

The first nerve to be given off from the plexus is the cruralis,

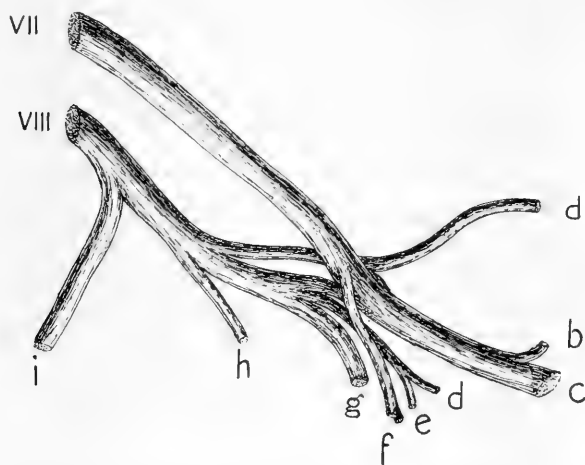


Fig. 7 Plexus from which the "aneurogenic" limbs are innervated, drawn from a graphic reconstruction projected to a horizontal plane. *VII*, r. abdominalis n. spinalis VII; *VIII*, r. abdominalis n. spinalis VIII; *a*, n. cruralis of primary limb; *b*, r. profundus posterior; *c*, n. ischiadicus of the primary limb; *d*, r. cutaneus femoris posterior of the primary limb; *e* and *f*, rr. accessorii cutanei femoris posteriores of the accessory limb; *g*, n. ischiadicus of the accessory limb; *h*, n. ilio-hypogastricus of the host; *i*, branch to the plexus lumbo-sacralis of the host.  $\times 67$ .

which originates from eighth nerve in a stout branch that runs more directly laterally than the main stem. At the point where it crosses the seventh it forms an anastomosis, giving off fibers to the latter, and perhaps, though not certainly receiving fibers from the same. It then runs a short distance anteriorly, arching around the ilium on the surface of the m. iliacus internus (Fig. 8). It gives off a branch to this muscle and then runs distally to the thigh for



some distance between the mm. adductor longus and pectineus, where it ends.

After giving off the cruralis and several branches to the abdominal walls, the eighth nerve passes abruptly in a lateral direction and intertwines in a complex manner with the seventh. From this

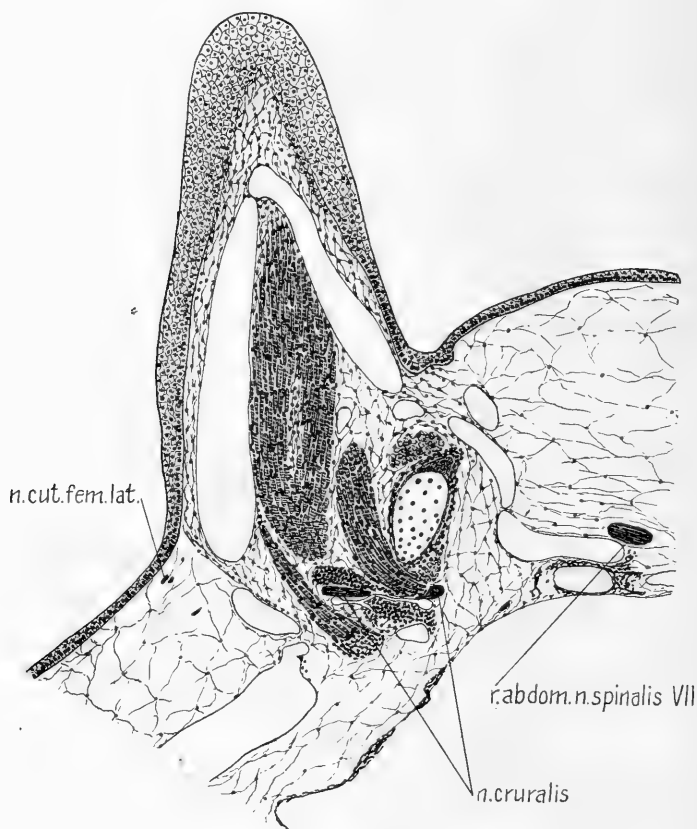


Fig. 8 Experiment I. Section through the thigh of the primary "aneurogenic" leg near the lateral surface, showing the entrance of the n. cruralis.  $\times 67$ .

plexus, the details of which are difficult to make out and to represent in the diagram, a number of nerves are given off. One runs to the secondary limb, becoming its n. ischiadicus (Fig. 10). Three other branches arise separately from the plexus, but before

finally dividing, run together only to divide again into a number of branches; several of these run down the thigh of the secondary limb and are distributed to the region over the *m. gracilis minor*, taking a course which is intermediate between the normal position

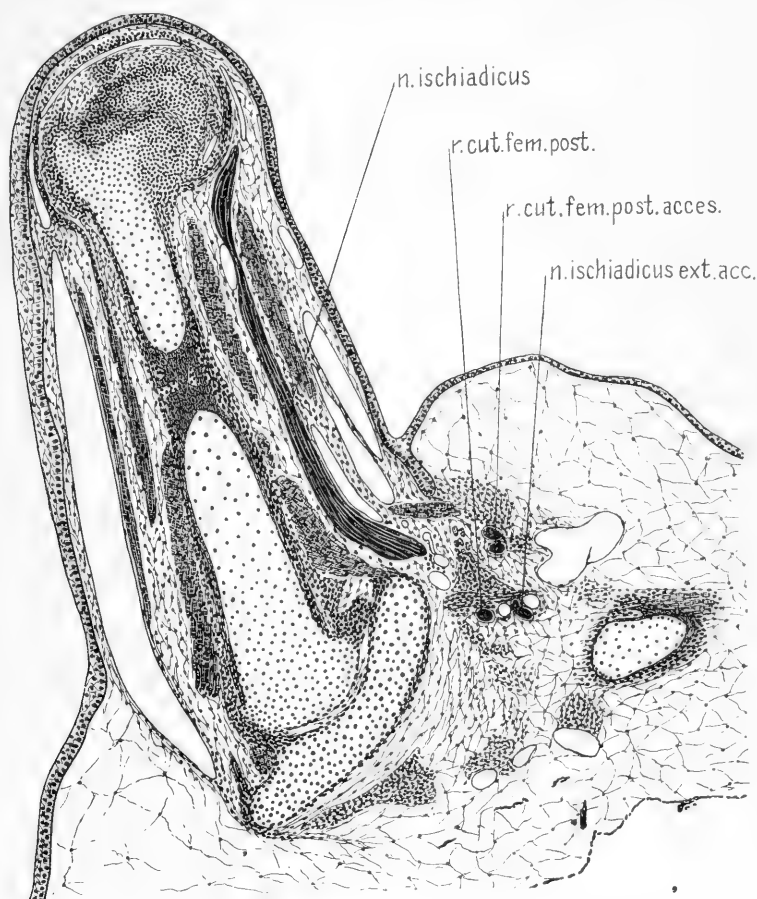


Fig 9 Experiment I. Section through the axis of the thigh of the primary "aneurogenic" limb. *r. cut. fem. post. acces.*, nerves supplying the region of the accessory limb usually innervated by the posterior cutaneous nerve. *n. ischiadicus ext. acc.*, sciatic nerve of accessory limb.  $\times 67$ .

of the *r. cutaneus femoris medialis* and *r. cutaneus femoris posterior*. One of the branches enters the primary limb as the *r. cutaneus femoris posterior*. The largest trunk from the plexus

becomes the n. ischiadicus of the primary limb, which may readily be followed to the knee in a single section (Fig. 9).

Within the primary limb the nerves may be clearly made out. The large sciatic nerve passes distally parallel to the m. ilio fib-

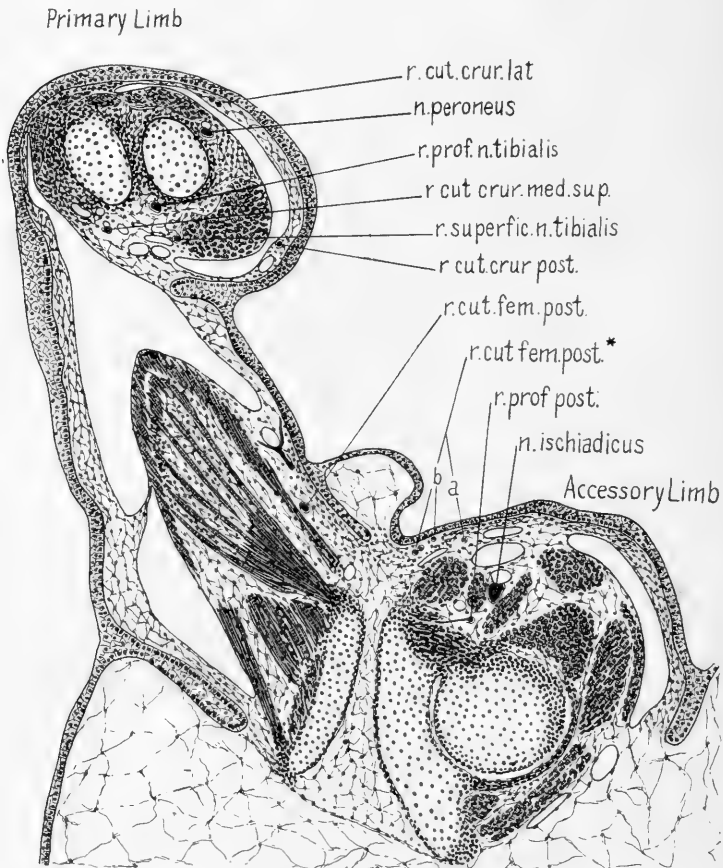


Fig. 10 Experiment I. Section through the thigh of the primary "aneurogenic" limb near the medial surface, showing also the secondary limb cut through the hip joint. \*a, r. cutaneus femoris posterior, derived from n. ischiadicus; b, accessory nerves derived directly from plexus.  $\times 67$ .

ricularis and gives off near its beginning the r. profundus femoris posterior. Before reaching the knee it divides into two subdivisions, the n. tibialis and the n. peroneus. The latter gives off a

nerve which follows the course of the r. cutaneous cruris posterior, a branch which normally comes from the tibial nerve. A little further down the r. cutaneus cruris lateralis is given off to the skin (Fig. 10).

Further down in the shank the peroneal nerve divides into a medial and a lateral ramus, the lateral being much the thicker of the two, especially at the point of origin. Lower down the medial ramus becomes stouter and a well defined branch to the m. tibialis anticus brevis is shown. The two peroneal nerves pass into the

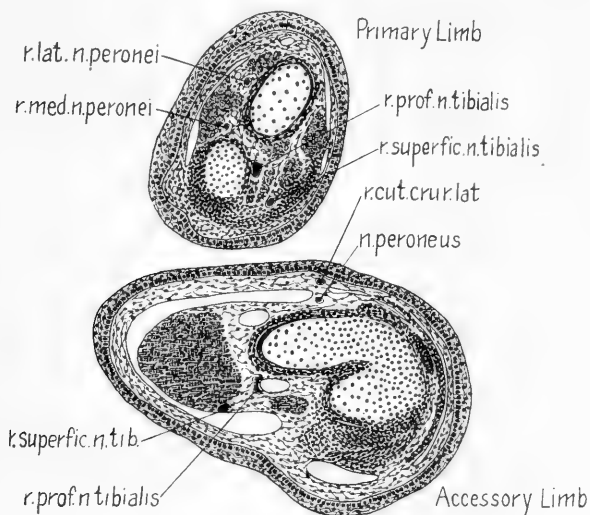


Fig. 11 Experiment I. Section through the two "aneurogenic" limbs; the primary limb is cut through the tarsus and the accessory limb just below the knee.  $\times 67$ .

tarsal region (Fig. 11). Below the middle of the tarsus they come together again to form the n. peroneus communis inferior. Before the dorsum pedis is reached a nerve is given off from this branch which may be traced out into the foot as the n. interstitiales dorsalis primus. In the foot itself the nerve breaks up into two other nn. interstitiales dorsales. One of these interstitial nerves, the third, could not be traced and all the nerves are very fine at this level. It will be seen from the above description that the n. peroneus has a normal distribution. The relations to the muscles and other

structures of the leg are also normal. The only anomaly observed was in the origin of the *r. cutaneus cruris posterior*.

The distribution of the *n. tibialis* is also the same as in normal limbs. First the nerve divides into a smaller *r. superficialis* and a larger *r. profundus* (Fig. 10). The latter then gives off a well developed trunk, the *r. cutaneus cruris medialis superior* which may be traced for some distance down the shank. The two rami of the tibial may be followed into the tarsal region and show normal relations to the muscles (Fig. 11). The *ramus profundus* passes into the *planta pedis* in the proximal part of which it breaks up into the four *nn. interstitiales plantares*. The manner in which these nerves arise is slightly different from the normal as described by Gaupp (Fig. 12). The *r. circumflexus* could not be traced.

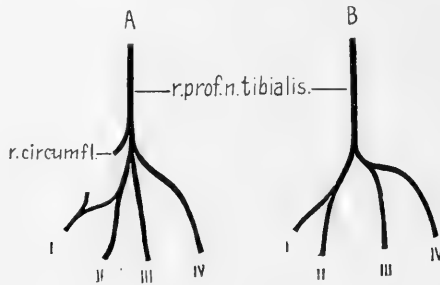


Fig. 12 Diagram of nerve supply to the *planta pedis*: A, according to Gaupp; B, as found in the primary "aneurogenic" limb in Experiment I. I, II, III, IV, *nn. interstitiales plantares*.

The accessory limb receives from the plexus a much smaller *n. ischiadicus* than the primary limb (Figs. 9 and 10). In addition to this it receives several short twigs that run subcutaneously along the inner posterior surface of the thigh, supplying an area of skin normally innervated by the *r. cutaneus femoris posterior* and to some extent by the *r. cutaneus femoris medialis*. The former nerve is small (Fig. 10), and arises in the normal way from the sciatic high up in the thigh. No traces of the *n. cruralis* have been found. The sciatic gives off a distinct though small *ramus profundus posterior*, though I have been unable to detect a *ramus profundus anterior*, which normally also arises from the sciatic.

In the lower part of the thigh the sciatic nerve divides as usual

into the peroneal and tibial nerves. The former gives off at the knee (Fig. 11) a ramus cutaneus cruris lateralis and continues down the shank whence it may be followed into the tarsus. This nerve is extremely difficult to follow and it is uncertain whether it divides into its r. lateralis and r. medialis. Certain it is that at the tibio-tarsal joint and further on only the r. medialis is present. This is, however, quite well defined and may be traced through

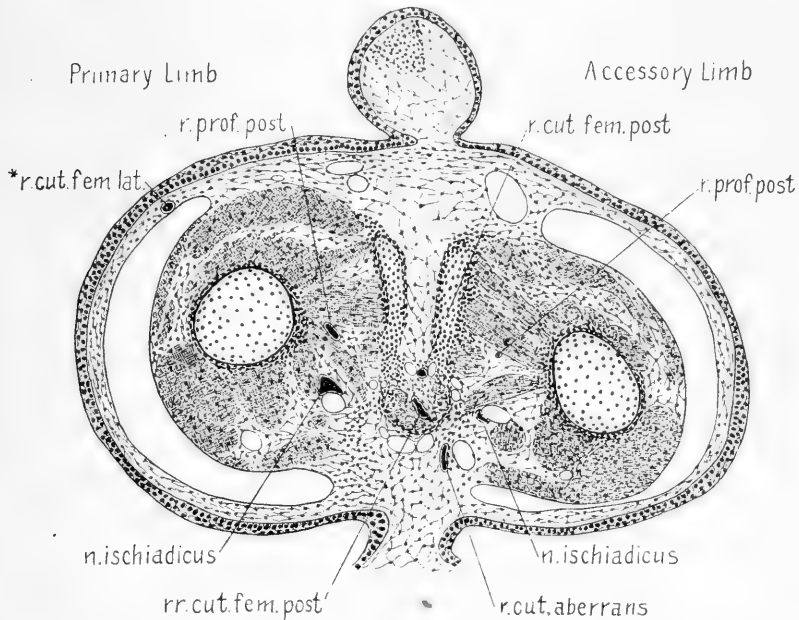


Fig. 13 Section through the two normal transplanted limbs in the upper part of the thigh. \*The ramus cutaneus femoris lateralis is some distance from its normal position.  $\times 67$ .

the tarsal region. I have not been able, however, to make out the nn. interstitiales dorsales.

The tibial nerve, soon after its origin, divides into a superficial and deep ramus of which, contrary to the normal condition, the former is distinctly larger (Fig. 11). This may be followed in its normal position through the shank and tarsus into the foot. The deep branch may be followed through the shank. At the tibio-tarsal joint it approaches very near to the superficial branch,

then it dips behind the tendon of the *m. tarsalis posticus* and may be followed for some distance through the tarsus. I have been unable to trace it out into the *nn. interstitiales plantares*.

The normal transplanted, or "euneurogenic" limbs (Fig. 13) receive nerves derived for the most part from the eighth spinal nerve. The seventh gives off a twig to the skin which becomes the *r. cutaneus femoris lateralis* of the primary limb; this nerve is, however, somewhat out of place. The main nerve (from the eighth) runs in between the pelvic cartilages of the primary and accessory limbs in three branches, one of which is considerably larger than the others. This gives rise to the sciatic nerves of the two limbs and to the crural of the secondary. The other two run distally between the pelvic cartilages, and emerge in the skin of the inner side of the two limbs becoming in each the *r. cutaneus femoris posterior*.

Into the primary limb the greater part of the fibers of the main trunk enter as the sciatic, which almost immediately gives off a stout *r. profundus posterior* which takes its normal course (Fig. 13). The sciatic may be clearly followed to the knee, breaking up into two main divisions, the peroneal and tibial. Below the knee the details of its distribution have not been studied.

The accessory limb receives nerves which are considerably smaller. The crural arises from the main trunk intended for the two limbs before this divides into the two *nn. ischiadici*. This nerve skirts along the surface of the *m. iliacus internus* and may be followed as far as the *m. pectineus*. As the *n. ischiadicus*, which is smaller than that of the primary limb, enters the accessory limb it gives off an aberrant branch which runs to the skin overlying the *m. glutæus magnus*. There is no nerve of this size in the normal limb at this place. Higher up, *i. e.*, before the sciatic actually enters the limb it gives rise to a small *r. profundus posterior* which passes ventral to the *m. piriformis* and may be traced along the dorsal surface of the *mm. gemellus* and *quadratus femoris*. It is not nearly so large as the corresponding nerve of the primary limb but it is normally situated with respect to the other structures of the limb.

The sciatic nerve may be followed through the thigh. It divides

as usual into the peroneal and tibial nerves. The former runs to the skin as the r. cutaneus cruris lateralis and I have been unable to find a continuation of the main trunk down the shank. The tibial nerve, as far as can be made out, runs entirely into the r. superficialis, the latter may be traced past the tibio-tarsal joint.

In comparing the innervation of the four transplanted extremities in the present case, it is seen that the primary "aneurogenic" limb has the most complete system of nerves, no important nerve being absent. Next in order comes the primary normal transplanted leg, in which, as far as studied, only the n. cruralis is lacking, though there is a nerve which probably represents the r. cutaneus femoris lateralis. The accessory aneurogenic leg is third; it lacks the crural nerve and some branches below the knee; the nerves could not be traced with certainty into the foot. The least complete system of nerves is in the accessory normal transplanted leg, where the sciatic nerve and its branches are much smaller than in the other limbs and no muscular branches are found below the knee. Oddly enough, however, there is a well developed crural nerve in this limb though it is entirely absent from the primary.

*Experiment II.*<sup>27</sup> For this experiment larvæ of *Bufo lentiginosus* were used. The procedure differed from the previous case, in that no normal limb was transplanted to the left side. Two nerveless hind limb buds were used; these were taken from a specimen from which the spinal cord had been removed one week before. The first limb transplanted was accidentally pushed through the wound into the body cavity. The second remained attached in the body wall. The larva grew rapidly and was preserved in Tellyesniczky's fluid thirty-five days after transplantation of the limb. As is readily seen in Fig. 6, the second of the transplanted buds has developed into a pair of hind legs, which are connected with the body by a short narrow stalk. Dorsal to these two legs, which are normally formed, there is an irregular mass, which sections show to have developed out of the bud that was pushed into the peritoneal cavity. Two hind legs are distinguishable in this mass but they are very irregularly developed

<sup>27</sup>Record number, Tr. Ext. 15.



and the sections are almost impossible to interpret. The description will therefore be confined to the other pair. While these limbs are further advanced in development than those in the previous experiment, it was apparent from the observation of the living specimen that they were not so well formed. They had a slightly atrophic appearance and were never seen to undergo even the slight twitching movements observed in the first experiment.

Sections show that a small nerve trunk, which arises from the eighth spinal nerve of the host enters the stalk which connects the limbs with the abdominal wall, and running between the two pelvic cartilages, is continued into the accessory or super-regenerated limb. This is all the more remarkable because no nerves could be traced into the primary limb. The nerve in the accessory leg follows the course normally taken by the n. ischiadicus. Above the knee joint it divides into two trunks, one running to the flexor and one to the extensor surface of the shank. Both of these are cutaneous nerves and correspond respectively in distribution to the r. cutaneus cruris posterior, which arises normally from the n. tibialis, and to the r. cutaneus cruris lateralis derived normally from the n. peroneus. No muscular nerves can be made out below the knee.

The imperfect innervation of the limbs in this case as compared with the previous one is due no doubt to less firm implantation into the tissues of the host.

*Experiment III.*<sup>28</sup> In this case a hind limb bud taken from a normal larva (*Bufo lentiginosus*) was implanted in the left side and one taken from a nerveless larva on the right. The spinal cord of the latter had been excised one week before.

Both limbs developed well and produced accessory limbs. The normal bud on the left produced a typical limb scarcely distinguishable from the primary. The nerveless limb produced an imperfect appendage, which in turn bore an accessory bud. The specimen was preserved forty days after the operation (Fig. 5).

The limbs derived from the normal transplanted bud receive a large nerve from the seventh spinal nerve of the host. Two

<sup>28</sup>Record number, Tr. Ext. 12.

small branches are given off to supply the primary limb. One follows the sciatic artery a short distance down the thigh, and is to be regarded as a rudimentary n. ischiadicus. The other runs to the skin of the thigh and corresponds in position to the r. cutaneus femoris lateralis. The secondary limb receives a much larger bundle of fibers. These run into a large n. ischiadicus, which however ends before it reaches the knee. It gives off in the upper part of the thigh a distinct r. profundus posterior.

The limbs derived from the nerveless bud also contain nerves. A branch from the seventh spinal nerve supplies them. This nerve runs first as a compact bundle. Just before passing the pelvic cartilage it becomes frayed out to some extent, but may nevertheless be followed nearly to the knee, giving off a r. profundus posterior.

The secondary limb is much less advanced in development than the primary but it receives a large branch of the above mentioned nerve, which runs into the thigh for a short distance in the position of the n. ischiadicus.

In this case, as is readily seen from the figure, all four of the transplanted limbs are considerably less advanced in development than in the first case described. It is possible that had the larva been kept alive for a longer time, the nervous system of the limbs would have become more complete. It is worthy of note that in this case the primary normal leg has the least complete innervation of all the four transplanted appendages.

*Experiment IV.*<sup>29</sup> This experiment, made upon *Rana* larvæ, differed from the others in that the limbs were transplanted to the back immediately behind the anterior lymph hearts. As before, a normal left bud was placed on the left side and a nerveless right on the right. The larva from which the latter was taken had lived nine days after extirpation of its spinal cord. Sections of this larva show that there are no nerves posterior to the vagus, the funicular fibers not even having grown out from the brain. The yolk is entirely absorbed except for a few granules in the intestinal epithelium.

<sup>29</sup>Record number, Tr. Ext. 11.

Each bud developed into but a single appendage, and neither of these were so far advanced in development as the limbs in the other cases described. Twenty-six days after the transplantation the specimen was preserved and afterward examined in sections. Both of the transplanted legs are innervated principally by the r. lateralis vagi. In the case of the normal transplanted limb on the left side a branch is given off from this nerve, which after skirting along a large vesicle, formed from the transplanted tissue, finally enters the thigh. Here it may be followed for some distance as the n. ischiadicus. In addition to this nerve a small branch from one of the spinal nerves extends out along the lateral surface of the thigh in the region normally supplied by the r. cutaneus femoris lateralis.

In the nerveless transplanted limb, which is cut more favorably than the other, the n. ischiadicus formed by the lateralis vagi may be readily traced to the knee. In this region it divides, one branch running to the skin where it may be followed some distance further. The other ultimately becomes lost in the mesenchyme. No traces of lateral line sense organs in the leg could be found. There are irregularities in the development of the cartilages in this limb and the muscles are scarcely differentiated at all so that the topographical relations are to some extent uncertain.

In comparing the above experiments it is seen that with a single exception all of the transplanted limbs contain nerves. There are great individual differences as regards completeness of innervation but in this respect the corresponding limbs in the different experiments do not occupy the same relative position. These features are expressed in the accompanying table. From this it is clear that the limbs which have been taken from nerveless individuals have fared rather better as regards innervation than the normal transplanted limbs have, and also that while the accessory limbs are less completely innervated in three cases, they are more completely innervated in two.

The most constant nerve is the ischiadicus, and as might be expected the proximal regions of the transplanted limbs are the most completely supplied. In general the cutaneous nerves are

more fully represented than the muscular. With regard to the former it may be pointed out that some variations in position and origin have been observed. These have been noted in both the "aneurogenic" and normal limbs and in the primary as well as in the secondary. It follows that we cannot discriminate between the different types of limb as regards their ability to acquire a normal nervous system.

*Table showing the relative completeness of innervation of the limbs in the individual cases.*

DESIGNATION OF TRANS- PLANTED LIMB	EXPERIMENT I		EXPERIMENT II		EXPERIMENT III		EXPERIMENT IV	
	Presence (x) or Absence (o) of Nerves	Rank as Regards Completeness of Innervation	Presence (x) or Absence (o) of Nerves	Rank as Regards Completeness of Innervation	Presence (x) or Absence (o) of Nerves	Rank as Regards Completeness of Innervation	Presence (x) or Absence (o) of Nerves	Rank as Regards Completeness of Innervation
Primary norm.	x	2			x	4	x	2 (?)*
Access. norm.	x	4			x	1		
Pri. nerveless	x	1	o	2	x	2	x	1 (?)*
Accessory nerveless	x	3	x	1	x	3		

\*It is uncertain which of these should be regarded as the more completely innervated.

### *Transplantation of Normal Limbs to Nerveless Regions*

The object of the following experiments was to test the power of peripheral nerve fibers to develop when entirely cut off from nervous connection with the centers. It has already been shown that both Braus and Banchi have failed to establish their claim that the nerves have this power, for in their experiments the nerves studied were contained in appendages that were implanted into regions where numerous nerves were present. The only crucial test of this question by means of the method of transplantation is to graft tissues containing developing nerves to the body of an individual in which nerves are entirely lacking, or at least to an extensive nerveless region.

While no difficulty has been encountered in obtaining tadpoles with extensive nerveless regions, it has heretofore been found

impossible to keep such specimens alive after the yolk is gone. Even when the brain and cranial ganglia are left intact so as not to interfere with the normal movements of the mouth parts and gills, the larvæ soon succumb, owing to their inability to move about and obtain food. During the course of my experiments in the spring of 1906 a method was devised for providing the cordless, and therefore paralyzed, larvæ with nurses. This is accomplished in the following manner: We start with embryos about 3 mm. in length, in which no nerves are as yet differentiated. After removal of the entire medullary cord caudal to the vagus region, a small piece is cut off the side of the belly of the embryo and a similar piece is taken from the opposite side of a normal embryo. The wound surfaces of the two embryos are then brought together and the embryos are held in place for an hour by means of pieces of silver wire, as described by Born, after which time they are permanently united. The further development of the pair takes place normally except as regards the direct effect of the wound healing, which brings about the formation of intestinal and vascular anastomoses between the two. In this way the normal component, which moves about and obtains food, is able to sustain the pair for some time. When the yolk is about absorbed the experiment is completed by transplanting to the nerveless component a limb bud taken from a normal larva of the same age or a little older. Such limbs contain, as already described,<sup>30</sup> the terminal twigs of nerves. The limb is grafted to the posterior part of the trunk a little dorsal and cranial to the natural hind limb, and in all cases was put on the free side of the body, *i. e.*, the side away from the nurse. The trunk region of the larva remains nerveless except that the *r. lateralis vagi* is present. There may be some extension of fibers from the nurse into the tissues of the nerveless component immediately adjoining the former, but only in one instance has a nerve been observed passing from the normal component to the opposite side of the other. This nerve passed under the notochord of the nerveless component after giving off twigs to the axial musculature and finally ended in the skin

<sup>30</sup>See p. 253.

of the opposite side some little distance from the transplanted limb. While it is necessary, therefore, to guard against contamination even in these experiments, still in all the cases but this one there is a very extensive nerveless region and the limb which is transplanted to this is far removed from the source of extrinsic nerves.

The outcome of these experiments was not altogether satisfactory because the limbs transplanted to the nerveless individuals in no case developed rapidly and hence even after the expiration of three weeks or more they are nothing more than mere knobs (Fig. 14). The natural hind limbs of these specimens are like-

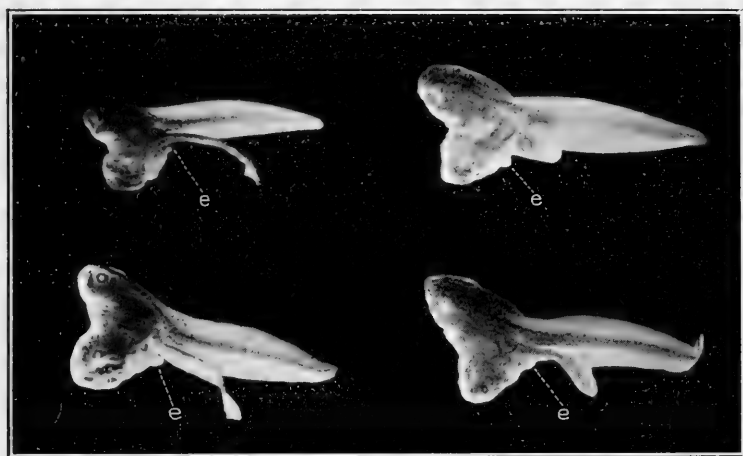


Fig. 14 Four nerveless larvæ, attached to normal nurses. *e*, transplanted extremity.  $\times 2$ .

wise poorly developed and it seems probable that the nutrition is insufficient to provide for a normal rate of growth. By modification of the method, I hope during the present season to obtain more conclusive results.

It will not be necessary to describe the individual experiments in great detail. Seven cases have been examined in all. The specimens were preserved two, three, five, seven, ten, twelve and fifteen days, respectively, after transplantation of the limb. In four of these no traces whatever of nerves could be found in the transplanted limbs or anywhere near them, although at the time

of transplantation the limbs did contain fine nerve twigs. The three specimens that do contain traces of nerves are those which were preserved two, seven and ten days, respectively, after the operation. In the first of these there is a short twig present which contains several Schwann cells and fibrillæ in a state of disintegration. The same is true of the ten-day specimen. In the seven-day specimen considerably more than the usual amount of tissue had been transplanted and this included part of several myotomes; hence this experiment is directly comparable to those of Banchi. In the transplanted tissue several structures are to be found which are undoubtedly degenerating nerves. One of these is a chain of cells without any visible fibrillæ; the others show fibrillæ in addition to the cells, though the former are indistinct.

The results of these experiments plainly indicate that there is no progressive development of the nerve after severance of its connection with the center. On the contrary there are rapid regressive changes, which in the majority of cases result in the entire disappearance of the nerves within a few days after they are cut off from their centers. The one case, in which the larger trunks were transplanted and which showed definite traces of the transplanted nerves after the expiration of ten days, may be taken as indicating that larger nerves will persist longer than the finer terminal twigs.

Two other series of experiments have been made for the purpose of testing in a more simple manner, the direct effect of the removal of the ganglion cell upon the growing nerve fiber. It was found that excision of the spinal cord in embryos of *Rana palustris* 8.5 mm. long results in the complete disappearance of the motor rami after a few days, and likewise the removal of the vagus ganglion in embryos 6 mm. long brings about rapid disintegration and early disappearance of the r. lateralis.<sup>31</sup> In these experiments sources of contamination from anastomoses were under control. The results are in entire accord with the results of transplantation of limbs just described, and they show that a

<sup>31</sup>These experiments were made in collaboration with Mr. Laurence Selling, who will report upon them later in full.

young developing nerve which has been deprived of its ganglion is doomed to early disintegration.

#### CONCLUSION

The immediate result of the experiments in transplanting extremity buds to normal individuals is to show that, as far as the acquisition of a normal peripheral nervous system is concerned, it is quite immaterial whether the bud, prior to its transplantation, has developed in connection with the central nervous system or not. It is likewise shown to be of no consequence, as regards its nerve supply, whether a limb develops directly out of the transplanted bud or whether it arises later as an accessory or super-regenerated appendage. These facts are in direct contradiction to the premises upon which Braus bases his support of Hensen's theory. The whole superstructure of his argument therefore falls to the ground, and it will be necessary to build entirely anew in inquiring into the bearing of the experiments upon the problems of the development of nerves.

It is clear that the experiments do not bear directly enough upon the point to decide satisfactorily questions of histogenesis, though they do throw important light upon the manner in which the peripheral distribution of the nerves is brought about. The original experiment of Braus, confirmed in the present study, shows that a normal limb bud, when transplanted to practically any region of the body of a normal tadpole, will acquire a system of peripheral nerves, which do not differ appreciably from the normal in their arrangement, and which are connected with the nerves of the region into which the limb is implanted, although in normal individuals the latter nerves may have no relation whatever with the limbs. This fact, though in other respects of cardinal importance, affords no solution of our problem because it may be interpreted in accordance with either the primary continuity or the outgrowth theories; either the nerves arise *in situ* out of structures present within the limb at the time of transplantation, or they grow in from the nerves of the host and are guided to their proper places of termination by the other structures within the limb.



Experiments which have already been reported by Lewis and by myself<sup>32</sup> are sufficient to decide in favor of the latter alternative. Destroy the nerve centers of an embryo no nerves ever develop. Transplant the centers containing ganglion cells to otherwise sterile (nerveless) regions, nerves will develop radiating from them, often following paths entirely unknown as nerve paths in the normal organism; in one case even the peritoneal cavity was bridged. Alter in the most profound manner the path normally taken by certain nerve fibers, at the same time leaving the nerve centers intact, fibers will, nevertheless, develop in connection with the center, following the normal direction of growth, though in strange surroundings. Again,<sup>33</sup> if the ganglion cells are removed after the nerves are partially developed, further development ceases and all traces of the nerve may entirely disappear; but on the other hand, as is well known, if the nerve is removed leaving the center intact, a new nerve is soon formed in its place. These facts show that the first essential for the formation of the nerve fiber is the ganglion cell. The only other condition, which, as far as known at the present time, is necessary, is that there must be a surrounding medium in the form of living tissue. There is no evidence, however, that any specifically formed or localized structures, essential to the formation of nerve fibers, are present within this medium.<sup>34</sup>

Hensen's theory supposes that the protoplasmic bridges connecting the various cells of the embryonic body, play this part.

<sup>32</sup>Harrison '06; Lewis '06.

<sup>33</sup>See p. 272.

<sup>34</sup>Recently Held has maintained, in accordance with Hensen's theory, that the outgrowth of the nerve fiber from the ganglion cell is only apparent and in reality is but a differentiation of preëxisting protoplasmic filaments found between the various organs in the embryo. "Entsteht also die Nervenleitung durch die Umwandlung von Plasmodiesmen in Neurodesmen" (op. cit., p. 188). A full discussion of this work will be deferred to a future communication dealing more especially with histogenesis. It may, however, be pointed out here that it is by no means certain that the plasmodiesms are not artefacts—products of coagulation (cf. Harrison, *Arch. f. mikr. Anat.*, Bd. 57, p. 421, and v. Lenhossék, *Neurologisches Centralblatt*, Bd. 26, p. 127). Nor can it be certain in consideration of the extreme minuteness of the structures in question, whether the fine filaments of the nerve process actually run into the fine protoplasmic threads or along them. At least it is very significant to find that Ramon y Cajal ('07), employing methods essentially similar to those of Held, nevertheless gives his full support to the outgrowth view.

Coming from a physiologist, the especial virtue of the theory, as might be expected, is physiological; it places the genesis of the permanent nerve paths upon the basis of functional adaptation; of all the numerous undifferentiated protoplasmic connections existing in the embryonic body, it is only those which function in conducting impulses that persist as permanent nerves; the remainder atrophy from disease. In refutation of this hypothesis it may be pointed out, however, that the functional activity of a nerve has no appreciable influence at least upon its early development. Amphibian embryos reared in a solution of acetone chloroform<sup>35</sup> acquire a perfectly formed nervous system, and one capable of normal functional activity, though during the whole period of their development up to the stage when the yolk is entirely absorbed, at which time the peripheral nerves are all well differentiated, no functional activity of the nervous system is manifested. Furthermore, in the transplantation experiments just referred to, a number of nerves were formed to which no conceivable function could be assigned, as, for instance, the funicular fibers which, after the removal of the medullary cord of the trunk, extend out from the brain and lose themselves in the mesenchyme.

It is precisely in this connection that the experiments in transplanting nerveless limbs are of great significance. A nerveless limb is taken from an organism that has undergone the greater part of its development after having been deprived of its spinal cord. As a consequence, no nerves are developed in the trunk region, and there is no evidence of nervous activity there, although in a normal individual during the same period, all of the important nerves, including those running to the extremities are visibly differentiated and for the most part are functioning. It cannot be supposed that the pre-nervous protoplasmic bridges, postulated by Hensen's theory, would be able to survive this long period of disuse, for, as experiments show, even visibly differentiated nerve fibers degenerate very rapidly after removal of their centers, often disappearing without leaving a trace in a much shorter time than that during which the nerveless individuals in question have been

<sup>35</sup>Harrison '04.

without their spinal cords. And yet when the limb of such an individual is transplanted to a normal larva it acquires a complete and normally arranged system of peripheral nerves just as a limb taken from a normal larva does. In other words, lack of functional activity, consequent upon the absence of nerve centers during a protracted and important period of development, does not in the least interfere with the later normal development of the nerves as soon as new nerve centers are supplied. These nerves, therefore, must be regarded as the product of the nerve centers alone.

This answer to the question being firmly established as correct, the cardinal importance of Braus' fundamental experiment becomes apparent, for, contrary to the conclusion drawn by its author, it shows that the structures contained within the limb must have a very important directive action upon the developing nerve fibers, in that they determine their mode of distribution. The manner of branching cannot possibly be predetermined in the ingrowing nerves themselves, because in the normal body these same nerves have an entirely different distribution. Let us picture to ourselves what probably takes place after a limb is transplanted. It is put into a region well supplied with nerves. The wound made for the reception of the bud involves without fail injuries to the nerves of the region. This stimulates the fibers to grow and in so doing some of them will come into contact with the cells of the transplanted bud, which at that time consists of a blastema of mesenchyme cells covered by epidermis but not visibly differentiated. As the bud grows into a leg and the blastema differentiates, the nerve fibers become arranged and segregated according as they are attached to the organs within the limb. In the limbs in normal position the development of the nerves must go on in the same way. Here too the nerve fibers reach the bud when it is still without visible differentiation. Contact with the cells contained within it being made at that time, the peripheral branches of the nerves are determined as the constituent parts of the limbs are segregated. The fact that any nerve in whose way a limb is planted is capable of giving rise to intrinsic nerves having perfectly normal arrangement, shows that the nerves themselves must be guided in the formation of their terminal ramifications.

This interpretation is in accordance with Nussbaum's law that the course of the nerve within the muscle is an index of the direction in which the muscle has grown.<sup>36</sup> In other words, the original point of contact between nerve and muscle persists as the entrance point of the nerve after growth is completed.

It is only necessary to suppose that action takes place at very short distances in bringing about first contact between the developing nerve fibers and the cells of the limb bud. Failure on the part of Hensen and the later advocates of his theory to realize this, has led to the great magnification of the difficulties which an outgrowing fiber would supposedly encounter in reaching its proper end organ. It is not necessary to imagine, as a number of writers do, that the growing nerve would have to wend its way through a labyrinth of differentiated tissues, extending from the hip to the toes, in order to reach its end organ, but merely that the nerve must grow independently as far as the base of the undifferentiated limb bud, the rest being provided by the development of the limb itself. The above interpretation calls nothing mysterious, nothing hypothetical into play. It is based solely upon known facts and does not postulate the existence of invisible and otherwise unknown structures. It is the only explanation that can be accepted in the present state of our knowledge. Moreover the variations in the distribution of nerves within normal limbs and especially the slight aberrations from the normal which have been noted in the position of some of the nerves in the transplanted limbs meet a ready explanation on this basis.

The foregoing suggests the consideration of certain meristic variations in peripheral nerves.<sup>37</sup> It has long been known, having been pointed out especially by Fürbringer, that the nerve plexus from which a limb is supplied might in two cases have a different metameric origin, and yet the nerves arising from the plexus might be distributed in the same manner in each. Gegenbaur, who, like Fürbringer, held closely to the theory that muscle and nerve form an inseparable unit, admitted the difficulty of satis-

<sup>36</sup>Nussbaum '94.

<sup>37</sup>This matter was brought up by Dr. McMurrich and Dr. Bardeen during the discussion of my paper at the Toronto Meeting, and has been fully discussed very recently by the latter (Bardeen '07).

factorily explaining such variations.<sup>38</sup> The interpretation of the transplantation experiments just given avoids this difficulty, for it brings out the fact that there are two main determining factors in the development of the innervation of a limb. The first of these is the position and extent of the extremity at the time of origin; this determines the source of the nerve supply. The second factor is the mode of segregation and growth of the individual structures of the limb, which determines the intrinsic distribution of its nerves. The experiments show that these two factors may be varied independently of one another. Variations in the position and extent of the rudiment of a limb, which may be assumed to occur frequently in nature, will, therefore, result in the ingrowth of different metameric nerves, and still the intrinsic distribution of their branches may remain constant, owing to the circumstance that the factors determining the latter operate in the same way regardless of the source of the nerves upon which they have to act. Both individual variations and specific differences in the metameric origin of limb-plexuses are naturally explained in this way.

#### SUMMARY

1 Limb buds of tadpoles, when transplanted to various parts of the body of normal individuals, develop normally and acquire usually a complete or partially complete system of peripheral nerves, which have normal arrangement and are connected with the nerves of the host supplying the region in which the limb is implanted.

2 The whole trunk region of an embryo may be made "nerveless" by cutting out the medullary cord posterior to the ear vesicle, just after closure of the medullary folds. Limb buds taken from such individuals and transplanted to normal larvæ behave exactly like the normal limb buds as regards the acquirement of nerves.

3 Accessory limbs, which frequently develop from transplanted buds by a process of super-regeneration, receive nerves either directly from the host or from nerve trunks running to the primary transplanted extremity. Sometimes the innervation of the acces-

<sup>38</sup> Die metamere Umbildung, wie sie sich als Verschiebung zeigt, bleibt damit ein Problem, dessen Lösung man sich vorläufig nur mittels der Hypothese nähern kann." Gegenbaur, *op. cit.*, p. 613

sory limb is more complete than that of the primary, though more frequently the reverse is the case.

4. It is possible to keep a nerveless larva alive for a period of a month by grafting it to a normal larva, which acts as a nurse. When a normal extremity bud is transplanted to such a nerveless larva, the nerve twigs contained within the former soon degenerate and no signs of progressive development of the nerves in such cases are to be observed. There is no evidence that an embryonic nerve can continue its development after its connection with the center is severed and prevented from being re-established. Cases which have been reported to the contrary are to be attributed to the presence of anastomoses.

5 The nerves are not formed *in situ* in the transplanted limbs but grow into them from the nerves of the host. Experiments which have previously been reported permit of no other conclusion and this is strongly reinforced by the experiments with nerveless limbs. Hensen's theory of primary continuity between nerve center and end organ is untenable, nor does functional activity play any part in the early development of the nerve paths.

6 Nerves reach the limbs, both natural and transplanted, when the limbs are in the earliest stages of their development and are composed of an undifferentiated blastema of mesenchyme cells. The intrinsic distribution of the nerves is determined by the structures within the limb, most probably at the time when the cells of the blastema segregate into the various definitive structures. This follows necessarily from the fact that any nerve which is led to enter the limb will assume the normal arrangement for that limb.

7 There are thus two important factors determining the innervation of a limb: First, its position and extent at the time of origin; upon this the *source* of nerve supply depends. Second, the structures within the limb itself; these fix the mode of distribution of the nerves.

8 These two factors are entirely independent of one another. Meristic differences in nerve supply of limbs, either between individuals or between species, which may exist without affecting the intrinsic distribution of the nerves, are to be regarded simply as due to variations in the original position and extent of the limb rudiments.

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# THE ENERGY OF SEGMENTATION

## AN APPLICATION OF PHYSICAL LAWS TO ORGANIC EVENTS

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## INTRODUCTION

The general purpose of this paper may be stated to be a philosophical, or at least a theoretical one. By this it is meant that the motive which has actuated it has arisen from an interest in two questions: first, as to just how different certain phenomena really are which appear to be very different, and, second, as to what the nature of the relation is between certain fundamental physical principles on the one hand and the manifold of concrete things on the other.

The first of these problems, to a solution of which an answer to the second is necessary, has, of course, a certain historical setting. Almost from time immemorial have discussions taken place as to the question, whether there is or is not a difference between life phenomena on the one hand and inorganic on the other, and if so, what this difference is. These have resulted, in general, in two positions, the one giving a mechanistic, the other a vitalistic school; the latter has, I think, in respect to details, varied from time to time, from period to period, more than has the former. However, it is not my purpose to justify this statement by presenting here the historical evidence for it; for whatever may be said of the vitalistic position and its variations, etc., the admission must be made concerning the mechanistic that, even up to the present, it has been held almost exclusively on the basis of general methodological principles alone. In very few instances has the attempt been successfully made to apply to that which the vitalist selects as distinctive and specific vital phenomena the same experimental methods, and through them the same physico-chemical principles as are used for inorganic phenomena. But even in these cases the admission must be made that such an application has been attended with success only for certain special and particular phenomena within the organism, and not for the organism as a whole. This result the vitalist gleefully points to and interprets as meaning that thereby there is eliminated only that which is purely physical and that the truly and distinctively vital still remains.

It is in connection with just this point, then, that the specific, yet philosophical, purpose of this investigation may be stated. It is,

first, to demonstrate that to the organism as a whole there can be applied upon a basis other than a general methodological one, namely, upon an experimental, metrical one, the same general principles of physico-chemical science as are used for the prediction, control, and explanation of inorganic events.

With this application successful in the particular instance which has been chosen for it, namely, the event of segmentation, then, secondly, as a valid generalization, I believe its meaning can be extended to other, perhaps to all organic phenomena, and the question made germane: Is it still correct to maintain the existence of a distinction between the organic and the inorganic, and, if so, in just what respect?

It will be recognized, then, that it is not only a fitting, but also a necessary preliminary to the carrying out of this program, that these principles, which, first, it is our immediate purpose to apply, which, second, have guided our experimentation, and which, third, must be considered in order to answer our major question, should receive a brief statement. These principles are the

#### FOUR LAWS OF ENERGY

Energy may be defined as that which does work,<sup>1</sup> and those which may be regarded as the four chief principles with respect to energy are as follows: Conservation, potential and potential difference, entropy, and invariability.<sup>2</sup>

The First Law is that of the conservation of energy. It may be stated in at least two ways:

First: In an isolated or closed system in which events are occurring or changes are taking place the energy-quantum remains constant; this continues to be the same all through the process. Change and constancy are thus in some way united.

In reality, however, no system is isolated, a fact which has very important consequences.

<sup>1</sup>Certain well-grounded objections have been made to this definition, but these we cannot consider here. Cf. *The General Principles of Physical Science*, by A. A. Noyes. pp. 73-4.

<sup>2</sup>A classification essentially similar to this has been given by Driesch in his *Natur-urteile und Naturbegriffe*. Leipzig, 1904.

By a *system* may be understood two or more species of energy coexisting within certain spatial limits. A system may, therefore, even if it is not to be isolated, be limited in this spatial sense. Accordingly, but only under certain conditions, which will be subsequently stated, the process taking place within such a system may consist in the change of a definite quantum of one energy-form into that of another. This, then, is *transformation*, and leads to the *second statement* of the principle of conservation.

In all energy transformations the quantum of one form disappearing reappears as a total, equal quantum of one or more other forms.

The First Law does not, however, state the condition for these transformations.

The Second Law does this. According to it, in its generalized form, first, each species of energy is the product of two factors, an extensity or capacity and an intensity or potential, these of course being different in the different species. Second, it is in virtue of intensities that events take place, but only under certain conditions: An intensity is a gradient and has direction. Accordingly, two intensities may be opposed, and meet, as it were, at a common point. Under this condition, they must be either equal or unequal. When the latter is the case there is, manifestly, a difference of intensity or potential; and if no third intensity be present, to supplement<sup>3</sup> the lesser one and to "make up" this difference, that is to "compensate" it, then something must occur; an energy transfer will take place from the quantum of the higher to that of the lower intensity. It is in this specific and exact form that the principle of efficient physical causation appears, thus giving us a "law of events."

This may be stated: Granted that all events in nature are energy transfers, for these the necessary condition is, not merely that there should be a difference between opposed intensities, but there must be at the same time an uncompensated difference. Then, with such an uncompensated potential difference existing, the process of doing away with it is forthwith initiated and takes

<sup>3</sup>Such supplementation is not additive.

place continuously; an energy transfer or transformation takes place in the direction of higher-to-lower potential. This process continues until the two opposed intensities are equal, *i. e.*, until equilibrium is reached.

Therewith, too, is that definition of *equilibrium* given, which applies to two opposed intensities. The other definition is in terms of compensation: A compensated potential difference is equilibrium.

In general, a system, supposing it to be isolated, is in *equilibrium* if all the intensities are constant, or, what is the same thing, if the quantity of each energy form which is free to transfer or transform is a minimum, *i. e.*, has the value zero.

However, this supposition is contrary to fact; an absolute and complete equilibrium is never really obtained.

It is this Second Law in particular which conditions the hypothesis through which my experimentation was guided, and the computations made. Accordingly, those of its details which subsequently come into consideration have been stated.

The Third Law, Entropy: This needs to be stated only because of its general bearing on the nature of organic processes and of its leading to the Fourth Law.

Its three aspects can be given briefly: First, most events are exothermic, *i. e.*, some of the energy of the system in which a transfer is taking place is always transformed into heat. Second, the intensity factor of heat energy, temperature, cannot be completely compensated; heat energy, therefore, spreads out, radiates, is dispersed. Third, just as the condition of this dispersion is a potential difference, so is the result a simultaneous tendency toward a uniform equalization of temperature, a condition under which heat energy is not further available; it is then "degraded." Because of these three simultaneous "tendencies" the entropy of the universe is said to increase. By virtue of them, "natural events" have an irreversibility, a definiteness of direction.

From the above statement it is clear that this Law is in some of its features only a part of the Second.

The Fourth Law: As really including, or as implied by, the three laws already discussed, and possibly therefore to be regarded

as the most general of all, there is that which may be and is sometimes called the Fourth Law, namely, that of invariability.<sup>4</sup>

The First Law expresses the quantitative identity or equality of those energy quanta, the conditions for whose causal action are expressed by the Second Law. According to these, in the transfer or transformation of energy, not only does the decrease in the quantity of one equal the total increase caused in one or more others, but the numerically expressed intensity fall of one is, in terms of a common measure, equal to the total numerically expressed rise in one or more others. For the figures for intensities can, of course, be added although intensities cannot be.

In general, *causa æquat effectum*; more cannot happen than there is sufficient cause for. Given, therefore, a definite causal quantum, one and only one effect, equal to that cause quantitatively, is brought about by it. In this respect, there is present, then, in the cause-effect relation, a singularity, a uniqueness.

In the Second and Third Laws there is implied a definiteness of direction in events, an irreversibility. First, this is given in the conditions for an energy transfer, *i. e.*, the existence of an uncompensated potential difference; and second, it has been found that most events are exothermal; they evolve heat energy, which, as it spreads out or dissipates, also comes to be more and more of a uniform temperature; the entropy increases.

That there is, then, *both* a singularity and a definiteness of direction in all events, as the three other laws show, constitutes a Fourth Law, which may be termed that of Invariability or Determinism in every particular, both qualitative and quantitative.

In general it is clear that the successful application of these Four Laws, or, primarily, of the First and Second, since these imply the Fourth, and the Third at least in part, will have a most important bearing on the question as to the character of organic events, etc., especially if this application be made on an experimental basis. But the necessary condition both for their application and for the computation of experimental results in accordance with them is their formulation and the demonstration of their relation to empirical laws. Accordingly, to this I now proceed.

<sup>4</sup>Driesch in his *Natur-urteile* develops a view similar in many respects to the one presented here.

THE EPITOMIZING FORMULATION OF THE FOUR LAWS, AND THE  
CONDITION FOR THEIR PRACTICAL APPLICATION

Already it has been emphasized, at least implicitly, that it is with systems, arbitrarily limited to a certain extent, that we are most intimately concerned in working out the meaning of, in applying, and in deducing the implications of the Four Laws. Now systems are by nature somewhat limited or closed; they are spatially marked off from their environment by a certain permanence of their configuration and proximity of their parts. It is with such systems that we have to do in experimenting with *living organisms*; in these the parts may be regarded as cells, or as organic structures within these, or as colloidal particles, etc., while from these parts there result creatively the qualities of whatever is for the problem at hand regarded as a whole.

Now, concerning any system which undergoes a change as a whole, three statements can be made, which are in agreement with the Four Laws, namely:

I. That by it a certain quantity of heat will either be taken up or given off.

II. That either it will do work or work will be done on it.

III. That the quantity of energy of the system will either increase or diminish.

In general, the change in the internal energy of the system is equal to the difference between the work done by the system and the quantity of energy added to it. This may be formulated as follows:

Let  $U$  = the internal energy of the system;

$Q$  = the heat energy taken up;

$A$  = any other energy added;

$W$  = the work done by the system; then

$Q + A = E$ , the total energy added.

Then, according to the First Law, and giving an analytic expression to it, after any change in the internal energy from  $U_1$  to  $U_2$ , as identical with which the system does work, the value of the change equals

$$U_2 - U_1 = E - W \quad [I]$$



Conversely, if work is done on the system, the value of this equation will be negative. Transposing equation [1] we get:

$$E = W + (U_2 - U_1) \quad [2]$$

The considerations leading to the formulation of the Second Law must be presented in some detail; for on its derivation in this form there hinges the demonstration that the computation of the energy of segmentation means the application of the Four Laws. Now it is well known, that, if we have a system by which, for example, a definite quantity of heat energy is transformed into work, while the system itself is not permanently modified, and granted that the process is taking place at different temperatures, the conditions which under it will give the maximum amount of work are: first, that there is no passage of heat by such direct processes as conduction or radiation; second, that there is no attendant irreversible process, such as friction; and third, and positive, that all the changes must take place under *equilibrium conditions*; that is, the intensity of the energy form undergoing change (within the system) must be compensated by an intensity externally applied and sensibly equal to it; this must simply be as great as is consistent with the occurrence of the change, since the work therewith produced is the greater, the greater the value of the compensating intensity. For the occurrence of the change, then, this external intensity need be less by only an infinitesimal amount than that within the system. These three conditions are, however, simply those which must be set up in order to bring about the reversibility of a process in a system; in fact, only on condition that the process is cyclical and reversible, will the system not be modified and the maximum amount of work be produced.

Now such a process will, as is well known, have four phases, the detailed analysis of which is, therefore, not required here. Their examination leads to the familiar expression

$$W = Q_1 \frac{T_1 - T_2}{T_1} \quad [3]$$

for the amount of work produced in such a cyclical process in terms

of the quantity of heat absorbed from the surroundings at the higher temperature, and the two temperatures involved.<sup>5</sup>

This equation is, now, a general expression of the application, to reversible cyclical processes such as the above, of the Second Law, which demands in this case that the same quantity of work be produced from a definite amount of heat by any reversible cyclical process whatever, taking place at the same two temperatures. It means that the maximum amount of work which, with the help of any system which is itself not permanently modified by the change, can be done by a definite amount of heat energy,  $Q_1$  at any definite temperature  $T_1$ , is *directly proportional to the difference between that temperature and any lower temperature with which the system can be brought into communication*. Accordingly

$$\frac{T_1 - T_2}{T_1}$$

may be called the "economic coefficient."

The principle expressed by equation [3] is of great importance both in general and for this present paper in particular; by means of it there can be determined the effect of temperature on the equilibrium conditions of a system, or on the value of the intensity factor of any energy form that may be involved; *or, if some other intensity be substituted for temperature, the effect of this could likewise be determined*.

Thus, if we consider a reversible change taking place in any system whereby a quantity of heat  $Q$  is taken from the surroundings at the temperature  $T$ , and a quantity of work  $W$  is produced in them, and if the same change of state takes place in the opposite direction and at a slightly different temperature,  $T + dT$ , and thereby a quantity of work equal to  $-(W + dW)$  is produced in the surroundings, there can be derived from equation [3], by substitution of the values:  $W_1 = W$ ;  $W_2 = -(W + dW)$ ;  $Q_1 = Q$ ;  $T_1 = T$ ; and  $T_2 = T + dT$ \*

$$dW = \frac{Q}{T} dT \quad [4]$$

<sup>5</sup>For the complete analysis and definition of this well-known equation, see Noyes, *Id.*, pp. 148-153.

\*For all the details of this, see Noyes, *Id.*, pp. 156-159.

Now the use which can be made of this equation is twofold, namely, theoretical and practical, with both bearing most intimately on our special problem. The theoretical will be considered first; it has to do with the generalization of the equation as expressed above. Here  $Q$  is the quantity of heat absorbed by the system when the change takes place in it under reversible conditions. But not only since heat is energy, but also since it is often impossible to measure this quantity of heat directly, there can be substituted for it, as equivalent to it according to the First Law, its value as obtained from equation [2] by putting, where  $A = 0$ ,  $Q$  in place of  $E$ ; we thus get

$$W + (U_2 - U_1) = T \frac{dW}{dT} \quad [5]$$

But, furthermore, just as  $Q = E$  (energy), so is  $T$  an intensity in equations [3], [4], and [5], and as these equations have been derived by considering reversible cyclical heat processes, so it is possible to derive analogous equations by considering reversible cyclical processes in which quantities other than, yet analogous to,  $Q$  and  $T$  are concerned. This could be done, for example, by considering electrical or mechanical changes.<sup>6</sup>

Thus, with  $U$  and  $W$  as before, if we let  $A =$  any other energy-form than heat, it is possible to get the equation, analogous to [3]

$$W = A_1 \left( \frac{I_1 - I_2}{I_1} \right) \quad [6]$$

in which  $I_1$  and  $I_2$  stand for any initial and final intensity, and likewise, analogous to [4],

$$dW = A \frac{dI}{I} \quad [7]$$

Accordingly, the possibility of such a procedure makes it quite evident that it is entirely permissible to make equation [5] of the most general form simply by replacing  $T$ ,  $Q$  having been eliminated, by  $I$  as standing for any intensity whatsoever; thus we get,

$$W + (U_2 - U_1) = I \frac{dW}{dI} \quad [8]$$

<sup>6</sup>Cf. Mach, *Prinzipien der Wärmelehre*, pp. 328-346.

Or, it is likewise for the same reason permissible to substitute in place of  $Q$  and  $A$  in equations [4] and [7]  $E$  as standing for any and all energy quanta added to the system under reversible conditions, thus getting

$$dW^e = E \frac{dI}{I} \quad [9]$$

which becomes, by substituting for  $E$  its value, found in [2],

$$dW = W + (U_2 - U_1) \frac{dI}{I} \quad [10]$$

from which equation [8] is again obtained.

Equation [8] is accordingly an epitomized expression of the First and Second Laws, since they alone have been used in deducing it, and therefore also, of the Third and Fourth to the extent, at least, to which these last have been found, in the preceding section, to be either further aspects or implications of the first two. The equation is, for these reasons, of the greatest importance.

In this equation  $W$  signifies the maximum external work produced by any reversible change taking place in any system at a constant intensity  $I$ ;  $U_2 - U_1$ ; is the accompanying change (increase) in the energy of the system itself;  $dW$  = the increase in the quantity of work produced when the same change in the system takes place at the intensity  $I + dI$ ;  $\frac{dW}{dI}$  is the potential coefficient, the extensity factor;  $I$ , the intensity; the resultant change in the energy of the system equals the product of the two.

Now, the above equation is worked out for changes taking place under reversible conditions; but since a change to be reversible must take place under *equilibrium conditions*, and it is upon these that the quantity of work produced is dependent, the above equation makes it possible to determine the effect of temperature or any other intensity on an equilibrium by calculating  $dW/dI$  when the other quantities  $W$  and  $U_2 - U_1$  are known. The equation will, then, have been applied to a concrete change, taking place of course according to some specific law, when the values for  $W$  and  $U_2 - U_1$  have been found by measurements and have been

substituted. For this, it is evident, the empirical law, according to which the maximum amount of work is given, must be known.

It is indeed just this method, the practicability of whose application to segmentation furnishes the experimental data or values for substitution in, and, therefore, for the computation, in accordance with, our epitomizing formula, of the energy-transfer involved in that event. The result is that this event, even with all its specific qualitative characteristics as a vital phenomenon, is brought under the Four Laws.

Now to determine  $W$ , it is necessary to know the conditions under which the change in state will take place reversibly, *i. e.*, to know the value of the intensities or resultant intensity, within the system, that must be *compensated* by an externally applied intensity in order to produce equilibrium and thereby reversibility. Then the product of this value, into the value of the change in the corresponding capacity factor, gives the value of the maximum amount of work produced by the change in state under consideration.

But, furthermore, if, during the occurrence of the change, the intensity-factor also varies, as does, for example, the pressure of a gas in expanding, then  $W$  is to be calculated from the corresponding integral, in this case,

$$W = \int_{v_1}^{v_2} p \, dv$$

To evaluate this, and corresponding integrals, the law of the variation of that intensity with the capacity-factor, that is, the functional relation between the intensity and extensity, during the change, must be known. This can be found only empirically and yet also, I think, only in accordance with and as implying the general principles expounded.

On the other hand,  $U_2 - U_1$ , is, if possible, to be determined by direct calorimetric or some other mode of measurement in calorie-equivalence units. But in some cases it is impossible to keep the two quantities  $W$  and  $U_2 - U_1$  distinct, and the measurements made (by compensation methods) furnish the basis for the computation only of the energy-transfer,  $E$ , as equal to  $W + (U_2 - U_1)$ ; that which is determined is, therefore, *the excess of the maximum*

work done over the simultaneous increase of the internal energy of the system; this excess is the energy withdrawn from the system, in accordance with which decrease work is done by it. Now, in the case of such an event taking place isopotentially and under equilibrium conditions, it is well known that both  $W$  and  $U$  (since  $U = W$  in accordance with the First Law) are determined simply by the initial and final states.

In the instance, for example, of the work being done against an outer pressure with an accompanying increase of volume,

$$W = \int_{v_1}^{v_2} p \, dv *$$

This becomes  $W = p (v_1 - v_2)$  with the pressure constant, or,

$$dW = d p (v_1 - v_2)$$

Substituting this value for  $dW$  in equation [8] and  $p$ , the intensity, for  $I$ , we get,

$$W + (U_2 - U_1) = E = (v_2 - v_1) p \frac{dp}{dp} = (v_2 - v_1) p = v (p_1 - p_2)$$

or, as is more usual, and since

$$\frac{dp}{p} = \frac{dT}{T}$$

$$E = (v_2 - v_1) T \frac{dp}{dT}$$

Equation [8] stands, then, in the relation of genus to a certain series of more special energy-laws as species; it, as has been shown, is derivable from them, but only by leaving out their differentia. Conversely, no one of these, in its entirety, can be deduced from it, but, for the discovery of their differentia, empirical investigation is necessary. Yet, in virtue of this relation, it is left quite possible, in fact made quite necessary, that the "differentia" and the "conferentia" should be entirely compatible, entirely capable of being conjoined. That is what is really demonstrated or done in the above substitution. That which is expressed in equation [8]

\*With the volume constant and the pressure changing,  $W = - \int_{p_1}^{p_2} v \, dp = v (p_1 - p_2)$

is really incorporate in any special energy-law as species, just as each special law is in turn incorporate in the concrete event.

Any determination of the value of an energy-transfer can be made only under the guidance of some knowledge or hypothesis as to what factors are concerned and also only through the application of some special energy-law such as the above,  $W = p v$ . But by the above substitution and derivation, as a result of which we could set

$$E = I \frac{dW}{dI} = v (p_1 - p_2)$$

it is shown that, when  $E$ , as the resultant energy decrease in an isothermal process, has been computed through the substitution of the values obtained by compensation methods for the initial and final pressures, with the volume constant, this determination is made in accordance with and through an empirical law which is a special case of equation [8]. The energy-transfer so determined is accordingly brought under the principles which equation [8] epitomizes.

Any possible criticism, therefore, that the procedure here has been in a circle, is no more valid than, or is as invalid as, it is in any case of the genus-species relation.

However, for the practical application of the fundamental equation to a change in a system under equilibrium conditions, it is necessary, in order to be able to make the requisite experimental measurements, not only to know what the general temperature conditions, etc., are, but also there must be an insight as to what other intensities and what extensity factors are directly concerned. Then and only then can our experimentation, consisting in methods of compensation, be adequately guided.

Such an insight, now, is to be obtained, in some cases at least, only by the forming of an hypothesis, a working point of view, which, of course, shall be based on as well founded and as complete data concerning the system in question as it is possible to get. Such an hypothesis must, then, be now developed as to the nature of those organic phenomena to which it is my purpose to apply the general principles which we have elucidated. The segmenting fertilized egg is the system whose changes are to be studied.

THE HYPOTHESIS

As indicated by practically all that which is at present known and accepted as to the constitution and action of "living matter" the egg of marine forms like the Echinoderms is to be regarded as a system of colloidal particles:

- 1 in solution;
- 2 with electrical charges, positive or negative;
- 3 they or their constituent molecules entering into chemical reactions;
- 4 this, however, sometimes, if not always, in the presence of ferments;
- 5 such particles being localized, to form the nucleus, the cytoplasm, etc.;
- 6 and with many energy-transformations taking place both within the system and between it and its environment.

Or, to state this another way, the ovum may be regarded as a *system* of energy-forms, chemical, electrical, volume, *i. e.*, osmotic, surface and heat. That each one of these is present in the egg can be demonstrated more or less directly; and for each and all of these together, it is to be granted—this is the hypothesis—that both the empirical laws of each form and the general laws are valid; between some two or more there may be from time to time equilibrium, at other times uncompensated intensity-differences, with events resulting therefrom. Accordingly, in agreement with the exposition above, the qualities of the organism, even granted that, as taken together, these are to be found together nowhere else than here, are either those which the specific energy-forms would retain if isolated, or they result in "creative synthesis" from just this coexistence of two or more forms, or both.

The hypothesis which has served as a working point of view, not only actually has been, but here, too, must now be developed gradually.

It is, first, and briefly, that the egg is such a system as is described above, namely, that upon it or by it work can be done; accordingly, that, in the end, all the "forces" or intensities are to be regarded as resolved into two, of which the one, as a resultant,



exerts a pressure from within outward on the cell membrane, thus tending to alter either the volume or the form of the egg, or both; while the other, to be identified with the "tension" of the cell membrane, opposes this. Accordingly, between these two opposed intensities there must be either equilibrium or an uncompensated difference in one direction or the other. Now this condition can be brought about, with the result that the externally directed pressure is greater than the internally directed, either by increasing the internal pressure, say, by a chemical splitting, etc., or by decreasing the membrane tension, or by doing both at the same time. Conversely, through any means by which the internal pressure is decreased, while at the same time the membrane tension is either kept constant or increased, a potential difference in the opposite direction can be established.

Omitting further details as to this aspect of the problem, it may be said that the above hypothesis was, in its essential features, presented in the author's paper on the Physics of Segmentation,<sup>7</sup> as descriptive and perhaps explanatory of the process of segmentation in general, and in particular of the efficacy of both artificial parthenogenetic and of normal fertilization, as well as of a large number of other experimental results and methods. Its success here, as I venture to deem it, together with its value as a working hypothesis by which there was made clear what the experimental basis for the application of general principles must be, rests as its only justification.

As against it, I am well aware that the criticism may be offered by, for example, the cytologist and morphologist, that segmentation is too complex for such a simplifying hypothesis to cover it; that it is the result, rather, of a much finer mechanism than is herewith assumed; or it may be objected by the physiologist that there are protoplasmic currents, or a disposition of electric charges, with resultant increased tensions at the equatorial plate, and a decreased tension at the poles, etc. Into the further discussion of these I do not need to enter, for, if my anticipatory statement of them is fair, these objections will have missed the point. For that which

<sup>7</sup>Biological Bulletin, vol. vi, 3, Feb., 1904.

they claim as to a finer structure and more detailed events is to be admitted, but that does not invalidate the hypothesis which I have advanced, that all these structures and forces *result* in two opposed forces, or can be looked upon as if they were two such resultant forces.

This is in accordance with the view taken of the purpose of an hypothesis, for example, by Boltzmann;<sup>8</sup> it is not that of seeing through (*durchschauen*) the mechanism of nature, but rather of giving a simple presupposition by which it becomes possible to "reckon" (*berechnen*) the phenomena of nature with the greatest closeness.

Regarding our procedure, then, as one justified both by precedent and by the results to which it leads, our hypothesis may be stated in a more complete and detailed manner as follows: It is, that normal fertilization brings about—perhaps somewhat gradually, and up to possibly the time of the metaphase—an increase in the resultant internal pressure directed against the cell membrane; that, as a result of this pressure-increment, there is established normally an uncompensated potential difference between the pressure which is directed externally from within and the opposed internally directed pressure of the membrane; that as the result of, or identical with the necessary transfer of energy under such conditions, segmentation takes place, with its accompanying increase of surface; that, however, by supplementing the pressure of the membrane, *i. e.*, by compensating this internal pressure-increment just before each segmentation, this event can be inhibited, and thereby the numerical value of the internal pressure, and its decrease with each segmentation determined. In this way, for each segmentation, equilibrium will be produced and, thereby, reversibility, so that with these data, together with the surfaces and especially the volumes of the various division-stages determined by measurement, a sufficient basis for the application of the appropriate empirical law, and the computation of the energy of segmentation in accordance with equation [8], will be

<sup>8</sup> Ueber die Methoden der theoretischen Physik, in Dyck, Katalog math. Modelle. Munich, 1892.  
Cited by Helm, Die Energetik. Leipzig, p. 338, 1898.

furnished. Accordingly this event will have been brought under the Four Laws of Energetics.

#### THE EXPERIMENTAL METHODS

The practical problem very evidently resolves itself into that of finding an efficient means of compensating, of establishing these equilibrium conditions. Since, now, by hypothesis, we are dealing with resultant pressures, it is clear that there can be used as a compensating means, only such an one as will give us a pressure which will supplement that of the egg membrane and be opposed in direction to the resultant pressure from within.

This means is found in the pressure of substances in solution, *i. e.*, in osmotic pressure. The law for osmotic energy (solutions) is, as is well known, in general the same as that for gases, namely,  $pv = RT$ .

It would appear, then, that, by using such an osmotic solution as has a pressure just equal and yet opposed to that increment in the outwardly-directed pressure which results from the fertilization of the ovum, the egg could be kept from segmenting, and a measure of this increment would be obtained. This can, in fact, be done, but with it two precautionary measures must be taken. For, first, since the normal medium of the eggs (*arbacia*) used is sea-water, and since it is known that the absence of this or of certain ions which it contains prevents segmentation,<sup>9</sup> it is necessary to retain this "ionic environment" in the experiments. But with this environment constant, since its effect on normal segmentation is also a constant one, this effect need not be taken into further consideration in the results of our experiments. In the second place, any inhibitory result coming from the chemical action of the solution on the ova must be eliminated; that of which exclusive use is to be made, in both experimentation and computation, is the "pressure effect" of the solution employed.

Now these two necessary conditions can be gained by the use of a solution of cane sugar in sea-water. For, thereby, first, although

<sup>9</sup>R. S. Lillie: Fusion of Blastomeres and Nuclear Division without Cell-division in Solutions of Non-electrolytes. Biol. Bulletin, iv, 4, March, 1903.

there are present in such a solution two osmotic pressures, due respectively to the salts in the sea-water and to the sugar in solution, and these pressures are additive and in that respect independent, the natural environment of the eggs is retained, and so, as constant, is eliminable; therefore will our concern be with the pressure of the sugar alone, as if it were isolated; and, secondly, any specific chemical action of the sugar solution can be regarded as eliminated, since it is shown from various experiments that for this urea, or glycerine, or, perhaps any other non-electrolyte, can be substituted.

The typical method of experimental procedure, of which the concrete results will be presented below, then, was as follows: A  $\frac{2}{3}$  mol. pure cane sugar solution in sea-water, the sugar having been previously carefully dried, was prepared; and sea-urchin eggs in excellent condition were selected and fertilized. Then, about ten minutes before cleavage might be expected to begin, these fertilized eggs were transferred to finger-bowls containing sugar solutions in a graded series of concentrations obtained by diluting definite amounts of the standard  $\frac{2}{3}$  m. solution with definite amounts of sea-water. Furthermore, the sea-water used in transferring the eggs was in each case accurately measured with a pipette and included in the computation, as was also the room temperature. Preliminary and control experiments had shown, too, that in the transferring of the eggs to these solutions, the period elapsed after fertilization was largely a matter of indifference; however, they were, in fact, transferred at the time stated in order to prevent the occurrence of cytolysis. During the experiment, especially while segmentation was taking place, it was necessary to make frequent and rapid observations both of "experimented" eggs and of control; for thus only could it be found which solutions were of a strength either just sufficient to inhibit the cleavage or just weak enough to allow it to proceed; observation was at this time necessary also for the reason that, since after some time nuclear division takes place in some cases, later observations as to the inhibiting point are, in general, not of as great reliability and accuracy as are those which are conducted as above. Now it is evident that, by such a method, there could be found with considerable accuracy a point

on one side of which the strengths of the sugar solution would permit segmentation to take place, on the other side, not; and that by repetition of this procedure with more finely graded solutions, this inhibition point could gradually be fairly definitely determined for each of the successive cleavages. This procedure was carried out by six successive experiments, with results which agreed so closely that further determinations seemed not to be necessary, even had the failure of material not made them impossible. The results, too, confirmed the hypothesis that for the inhibition of each successive segmentation a lower pressure was required than for the preceding. The numerical value thus obtained for any two such pressures before and after a cleavage, since each pressure is a measure of the opposed resultant pressure from within, gives the basis for the computation of the energy-transfer involved in that cleavage.

It is evident, then, that the egg is here regarded as a *system* in which, acting from within outward against a certain infinitesimal surface ( $s$ ), there is a resultant pressure  $p_i$ ; and that the opposed pressure of the membrane before fertilization is just sufficient to *compensate* this. Let the internal force  $F_i = p_i s$ . Furthermore, it is conceivable, that, as a result of fertilization a chemical splitting shall take place, by which  $p$  is increased; as a result of this increase, something must change, *perhaps* the volume. It might be supposed, then, that ( $s$ ) would be thereby displaced, for an infinitesimal distance,  $dl$ , giving, therefore, an infinitesimal increase of volume,  $s dl = dv$ ; the work,  $dW$ , done by the system in such a change, *i. e.*, the change in its volume energy is, therefore,

$$dW = -dE = F_i dl = p s dl = p dv \quad [11]$$

Now, any external force  $F_e$ , directed against the internal force  $F_i$  and sufficient to prevent such a change, must be equal to  $F_i$ ; or, since it acts on the same surface ( $s$ ), and this is constant, the external pressure equals the internal.

Any finite change is, then, the integral of all the infinitesimal changes, that is,

$$W = E_1 - E_2 = \int_{v_1}^{v_2} p dv \quad \text{or} \quad - \int_{p_1}^{p_2} v dp \quad [12]$$

With the pressure constant during a change in volume, this becomes

$$W = p (v_2 - v_1)$$

or with the volume constant and the pressure changing,

$$W = v (p_1 - p_2)$$

Furthermore, in this connection it must be known whether, during the change, the temperature remains constant or not, *i. e.*, whether the event be isothermal, or adiabatic.

That that event of segmentation which we are considering, must, under its normal conditions be isothermal, is, I think, a safe assumption and one universally agreed to; it is in accordance both with that which would seem necessarily true, namely, that the temperature of the organism must, in the large amount of seawater by which it is surrounded, remain essentially constant, and with what is known of the temperature of organisms in general.

Do, however, the surface and volume remain constant? To decide this question, first, measurements of the diameters of a number of eggs in the one-cell stage were made with the ocular micrometer; in doing this as perfectly spherical and as typical individuals as possible were selected; then, likewise, the two axes of each cell in the two-cell stage, where each cell is an oblate spheroid, were measured. This was not done for the later stages, owing to the many difficulties which would have been encountered in selecting axes and getting formulæ for computative purposes. Of these numerical results averages were taken, and, by the use of the appropriate formulæ, the surface and the volume were computed. This gave the result, that, while the *surface undergoes a marked increase*, as accompanying each segmentation, the *volume has remained constant*.

Since, then, as accompanying each cleavage, a change of pressure is demonstrated by the compensation method, and there is no change in volume as shown above, and the event is isothermal, the equation

$$W = E_1 - E_2 = - \int_{p_1}^{p_2} v \, dp \text{ becomes here } E = v (p_1 - p_2)$$

Now just as it has been found previously that

$$E = W + (U_2 - U_1) \quad [2]$$

and that

$$W + (U_2 - U_1) = I \frac{dW}{dI} \quad [8]$$

and (p. 295), when it is known that we are dealing with initial and final pressures, with volume constant in an isothermal event, that

$$E = v (p_1 - p_2)$$

and that, therefore,

$$W + (U_2 - U_1) = v (p_1 - p_2)$$

so here it is impossible to determine separately the two quantities,  $W$  and  $U_2 - U_1$ ; rather, the computation in accordance with the values found for  $v$ ,  $p_1$  and  $p_2$  gives only a resultant, namely, the excess of the maximum work done by the system over the simultaneous increase in its internal energy. This computation, furthermore, has already been shown (p. 295) to be in accordance with and a special case of our general formula [8], and, therefore, of the Laws which it expresses.

This relation can be further demonstrated as follows:

In equation [8] substitute for  $I$  the intensity factor  $p$ , and for  $W$  the product  $p v$ , found in [11]. Then, with  $U_2 - U_1 = 0$ , as in the case of the expansion of a perfect gas, we get

$$p v = p \frac{v dp}{dp} = p v \quad [13]$$

The equation comes to identity, as is necessary. Nor would this demonstration be altered in the case in which  $(U_2 - U_1) > 0$ ; for  $(U_2 - U_1) = E = W$  in accordance with the First Law, and so might be set in equivalence with  $p v$ , and this substituted, with the result an identical equation as before.

By the results of the computation in accordance with the results obtained from measurement, namely, that an original increase of pressure following fertilization and a decrease of this following each cleavage take place, while the temperature and volume remain constant, the quantitative value of the resultant energy-changes will, then, have been determined. But, in accordance

with this mode of determination,  $W$  and  $U_2 - U_1$  will not have been kept apart; accordingly it is quite possible that during the event of cleavage heat is absorbed from the environment, that the internal energy will have increased by a certain amount. However, to determine the exact value of such an increase would seem to be impossible. Yet experiment makes clear that there is a change in pressure. To what is this due? Equation [8] shows that in general such a change must be due to the fractional change in some other intensity; for, with  $U_2 - U_1 = 0$ , and  $W = pv$ , by substitution in [8] we get

$$pv = I \frac{p dv}{dI} \quad \text{or} \quad \frac{d p}{p} = \frac{d I}{I}$$

That this change should be the result of a change in temperature is impossible if the view is correct that this last intensity is constant; yet correlative change there must be, and the evidence is that this is in the chemical conditions.

#### RECORD OF EXPERIMENTS

The following is an epitomized record of the experiments performed,<sup>10</sup> showing what solution-concentrations were sufficient to inhibit the earlier cleavages; the results will be found to show a fairly close agreement. The figures given here are simply those of the concentration, obtained in each case by starting with a certain number of cc. of a  $\frac{2}{3}$  m. sugar solution in sea-water for each lot of eggs transferred, and then diluting this amount with a certain measured amount of sea-water, plus the  $1\frac{1}{2}$  cc. of sea-water necessary for transferring the fertilized eggs. The pressures thus obtained are here not reduced to atmospheres; that is done only for the typical experiment in which the computation is carried out completely. In all of these the eggs in the control segmented very uniformly and well.

Experiment I, July 15: First segmentation stopped in sol. 10 cc. sugar sol. plus 11 cc. sea-water plus  $1\frac{1}{2}$  cc. s. w.,<sup>11</sup> and in

<sup>10</sup>The experimentation was done in the Marine Biological Laboratory at Woods Hole, in the summer of 1905.

<sup>11</sup>s. w., sea-water.



all stronger; continued in all weaker solutions. Second cleavage stopped by 10 cc. sugar sol. plus 13.75 cc. s. w., plus  $1\frac{1}{2}$  cc.; the third by 10 cc. sugar, plus 15 cc. s. w. plus  $1\frac{1}{2}$ ; the fourth, by 10 cc. sugar plus 16.5 plus 1.5 cc. s. w.

Experiment II, July 21: Made for purposes of refinement; found to be confirmatory of preceding.

Experiment III, July 22: First segmentation stopped by 15 cc. sugar plus between 16 and 18 cc. s. w. plus  $1\frac{1}{2}$  cc.

Experiment IV, July 22: Inhibition point found to be between 16 and 16.5 cc. s. w. plus 15 cc. sugar plus  $1\frac{1}{2}$  s. w.

Experiment V, July 24: First cleavage stopped by 15 cc. sugar plus  $\frac{1}{2}$  (16 plus 16.5) cc. s. w. plus  $1\frac{1}{2}$  cc. This means that the (16), and all stronger, stopped, while the (16.5) and all "up" from this "allowed to proceed." This method of taking the "intermediate point" was subsequently adopted in each case. Second segmentation stopped by 15 cc. sugar plus  $\frac{1}{2}$  (20.5 + 21) s. w. plus  $1\frac{1}{2}$  cc.; third, by 15 plus  $\frac{1}{2}$  (21.5 + 22.5) plus  $1\frac{1}{2}$  cc.

Experiment VI, July 27: Temperature 23.5 C. Fine lot of eggs. Fertilized at 11 a. m.; repeatedly and frequently observed up to 3 p. m.; observations all confirmatory. First segmentation stopped by solution 15 cc.  $\frac{2}{3}$  mol. sugar sol. plus 16.75 cc. s. w. plus 1.5 s. w.; second by 15 cc. sugar plus 20.75 plus 1.5 cc. s. w.; third by 15 cc. sugar plus 21.5 plus 1.5 cc. s. w. Control; over 95 per cent of the eggs segmented uniformly.

For the purposes of the computation to be made, the surfaces and volumes of each stage must be found. This was done as follows. In the one-cell stage the typical or "modal" sea-urchin egg is approximately spherical; accordingly the diameters of as large a number as the period of 55 to 60 minutes elapsing before the first segmentation allowed were measured by means of an ocular micrometer; from these data, widely divergent values being excluded, the average diameter was found, and the surface and volume computed from well-known formulæ. In the two-cell stage the typical form is that of two oblate ellipsoids; the long and the short axis of each ellipse was accordingly measured for a number of eggs, the average for each taken, and the surface and volume computed. Difficulties in doing this for the four and eight-cell

stages were foreseen, but the results obtained for the first two stages showed that the volume after the first segmentation was the same as before it; it could, therefore, be assumed to be constant during the second and third and even subsequent stages, especially since general observation makes no change manifest. On the other hand, the increase of surface which was demonstrated to have taken place was shown to be not of direct significance in the computation made of the energy of segmentation.

These measurements of diameters were made in Experiments V and VI on eggs taken, of course, from the control.<sup>12</sup> But it is evident that the numerical results thus obtained are in any complete computation to be combined with those obtained from the use of the inhibiting solutions on eggs of the same lot. Accordingly, it is the result of the complete computation from all the necessary experimental data, as taken in Experiment VI, that is presented below; and in connection therewith it may be remarked that, as between method and numerical result, it is the former rather than the latter that I would have regarded as the more worthy of emphasis. If the question be raised as to the accuracy of the numerical result, this can be estimated by considering the sources and probable limits of error introduced both by the method of compensating and by the fact that one is observing a "group" of eggs and must adopt the expedient of taking averages, etc.

#### OBSERVED AND COMPUTED RESULTS IN EXPERIMENT VI

Data: Segmentations stopped, the first by 15 cc.,  $\frac{2}{3}$  m. sugar sol. plus 16.75 plus 1.5 cc. s. w. at 23.5° C., etc.

Now, it is well known that osmotic solutions in general follow the law for gases; and it is held, too, that there is no dissociation in a sugar solution. Accordingly the pressure of a mol. sugar sol. at 0° C. is 22.4 atmospheres.<sup>13</sup>

<sup>12</sup>Miss Evis Berry kindly assisted me in the experiment in this way.

<sup>13</sup>An atmosphere is that unit of pressure which is exerted by a column of mercury 76 cm. in height of a density 13.596; this equals in C. G. S. terms 1013300 dynes, *i. e.*, the pressure of such a column of mercury per sq. cm. The egg has of course an area which is only a small fractional part of a square centimeter.

Making the correction for a room temperature of 23.5 C. in accordance with the formula,  $p_t = p_o (1 + .00367 t)$ , the osmotic pressure of the above diluted solution, *i. e.*, the pressure sufficient to inhibit the first segmentation and therefore equal to the resultant internal pressure, is 7.32 atmospheres.

For the second segmentation, the numerical value of this inhibiting pressure, as computed in a similar manner from the recorded figures above, is 6.53 atmospheres, and for the third, 6.40 atmospheres.

#### MEASUREMENTS OF DIAMETERS AND AXES

Average diameter of 20 typical eggs = .072 mm.; radius = .036.

Area ( $4 \pi r^2$ ) = .0164 sq. mm.

Volume ( $\frac{4}{3} \pi r^3$ ) = .000214 cu. mm.

Two-cell stage: each cell an oblate ellipsoid. Average axes of 20 typical segmented eggs:

Long axis ( $d'$ ).....068 mm.

Short axis ( $d$ ).....039 mm.

Area  $\left( \sqrt{\frac{d^2 \times d'^2}{2}} \times \pi d' \right) = .0118$  sq. mm.

for each cell; for both .0236 sq. mm.

Volume ( $\frac{4}{3} \pi d d'^2 \times 2$ ) = .000206 cu. mm. for both cells together.

From these values it is evident that, whereas the area has increased by .0072 mm., the volume has remained the same.

It would now seem as if the data were at hand whose numerical values could be substituted in the "work integral"

$$W = - \int_{p_1}^{p_2} v \, d p$$

which becomes  $W = v (p_1 - p_2)$  when the volume remains constant, as in this case. However, before doing this, the question must be answered, as to what may be the value of that pressure which is due to the tension of the surface film or membrane of the developing egg. For it might seem that the resultant internal pressure before and after each segmentation was equal to, not alone the opposing osmotic pressure of the surrounding sugar sea-water

solution, but, rather, to this plus the pressure of the surface film or membrane. Accordingly, the numerical value of this must be found, that it may be known whether it is significant for our computation or not.

The formula by which this pressure due to the tension of the surface,<sup>14</sup> if this be only a film like the surface of a drop of water, may be computed, is

$$p = \frac{2 t}{r}$$

in which  $t$  is the coefficient of surface tension and  $r$  the radius of a sphere. This  $t$  is determined from the capillary action of a fluid in accordance with the formula,  $t = \frac{1}{2} g r h D$  ( $g$  = action of gravity,  $r$  = radius of tube,  $h$  = height to which the fluid is drawn up,  $D$  = the density).

Pfeffer<sup>15</sup> gives this coefficient as .01 g. cm. in relation to that of water as unity. Since other determinations are lacking, I made use of this, although, of course, it must be admitted that this coefficient might vary greatly with different kinds of protoplasm.

Substituting this value in the formula,

$$p = \frac{2 t}{r}$$

$$p = .0055 \text{ atmos. pressure}$$

For the two-cell stage, with each cell an oblate ellipsoid, the formula is more complicated: here

$$p = \frac{t \cdot 4 \pi \left( c \frac{a^2}{\sqrt{a^2 - c^2}} \tan^{-1} \frac{\sqrt{a^2 - c^2}}{c} \right)}{\text{area}} *$$

( $a$  = long axis,  $c$  = short)

<sup>14</sup>The best treatment of the general problem of surface tension, etc., which I have found is M. Heidenhain's *Die allgemeine Ableitung der Oberflächenkräfte*, etc., in *Anatomische Hefte*, erste Abteilung, vol. xxvi. Wiesbaden, 1904.

<sup>15</sup> *Plasmahaut u. Vakuolen*, Abhandl. d. Math.-phys. Kl. d. Sächs. Ak. d. Wis., 16, 185 (1891); cited by Höber, *Physikalische Chemie der Zelle u. Gewebe*, s. 38; Leipzig, 1902. This is the only determination I have been able to find.

\*For this formula I am indebted to Dr. C. R. MacInnes, of Princeton University.

Substituting, we get

$$p = .0063 \text{ atmos.}$$

Although, therefore, it appears from this that there has been an increase in the pressure which would result from the curved surface of the egg were this a film, it is also evident that this is of insignificant value in comparison with the values, 7.32, 6.53, 6.40 atmos. found for the inhibiting solutions. It falls "outside the limits of error," and is, therefore, to be neglected in the application of the "work integral."

The fact, however, that this pressure has such a small comparative value, results, evidently, from the substitution of .01 as the coefficient of surface tension of protoplasm. The acceptance of this value is, of course, purely gratuitous; but if it be approximately correct for the protoplasm of the sea-urchin egg, then the resulting small value of the pressure of the surface on the basis of the assumption that this is a film proves this assumption to be incorrect, and indicates that there must be a membrane, differentiated from the cytoplasm, to oppose the relatively high internal pressure as indicated by the strength of the solutions requisite to inhibit segmentation.

There has been demonstrated, then, experimentally, an increase of 7.32 atmos. in the "resultant" pressure, as brought about by fertilization and the process following it up to the time of segmentation. As a result of these, the egg normally cleaves; it changes form, and it is now shown experimentally that as it does this the internal pressure therewith decreases; without fertilization these events do not take place.

For the early segmentations, then, there are numerical data at hand from which the resultant energy change can be computed in accordance with the "work integral"

$$W = - \int_{p_1}^{p_2} v \, d p$$

which becomes, when the volume is constant,

$$W = v (p_1 - p_2)$$

Substituting in this the numerical values obtained for pressures and volume, we find that there has taken place, as a result of fertilization and processes subsequent to this, an increase in the energy of the egg of

$$7.32 \times 1,013,300 \times .00000021 \text{ cu. cm.} = 1.567 \text{ ergs;}$$

that, analogously, after the first segmentation, the energy is

$$6.53 \times 1,013,300 \times .00000021 \text{ cu. cm.} = 1.399 \text{ ergs.}$$

This means, that, as involved in or as identical with the first segmentation, there has been a resultant energy decrease, therefore, of .168 ergs; or that it has taken this amount of energy, about  $\frac{1}{9}$  of the total increase resulting from fertilization, etc., to bring about this cleavage.

As bringing about the second segmentation, we find by substitution:  $(6.53 - 6.40) \times 1,013,300 \times .00000021 = .028$  ergs of energy to have been involved.

#### CONCLUSION

This completes the computation based on the measurements taken in Experiment VI. It could, of course, also have been made for some of the other experiments, and, had our purpose been to determine as accurately as possible the numerical value of the energy of segmentation, then a large number of both experiments and computations would have been necessary, in order from these to get a mean result. But it has been not the numerical result but rather the method, that is, the practicability, on an experimental basis, of applying the "work integral" and so the other equations of which it is a special case, that has seemed the more important and been deemed worthy of emphasis. Thus would I forestall the point of the possible criticism that the numerical results themselves are meager, and that they have been found for only two segmentations on data obtained in one experiment. For, while these are the facts, nevertheless, on the other hand, Experiment VI and its results can be regarded as typical of further possibilities, while on the other the limitation to two segmentations

may be regarded as due simply to certain difficulties in experimental procedure which, of course, further refinements may overcome.

Accordingly, I shall consider that that which was my immediate purpose, namely, the application to an organic event of the same general principles as are applied to inorganic events, has resulted successfully, and that thus a basis is furnished for answering the other questions which were propounded at the beginning of my paper.

However, before that is done, an interpretation must be made as to just what the results obtained show as to the character of the energy-transfer which is involved in each cleavage. Here the principles stated in our introduction and developed in our formulation must guide us.

In answering this question it must be said, in accordance, first, with what was shown as to the conditions under which our measurements must be taken, and, second, with the hypotheses formed as to the forces, etc., in the egg as a system, that the numerical values obtained for the energy-transfer in the two cleavages are the *measure*, first, of the difference between the energy decrease and its simultaneous increase,  $E = W + (U_2 - U_1)$ , during the event of cleavage; and, second, of the resultant, in energy-terms, of all those subsidiary processes and changes, morphological and otherwise, which contribute to the event; some of these must be identical with  $W$ , others with  $U_2 - U_1$ ; if there be any processes which do not so contribute either directly or remotely, then, of course, they are not included in this resultant.

It is evident, then, first, that the result obtained allows for the possible increase in the internal energy of the ovum by the absorption of heat, or other energy, though probably only the former, as simultaneous with a decrease in accordance with which work is done; and, second, that our result gives the measure, not of the entire energy of the cell, but only of that which, as an excess of the energy "lost" over that gained, is identical with the energy of cleavage.

What, now, is the character of the energy-form in which there is this resultant decrease? To this the answer is indicated, first,

by the hypothesis formed that in cleavage we are dealing with "forces" which are efficacious only as *resultant pressures*, and, second, by the nature of the factors actually determined by measurement, again pressures, that it is the "volume energy" which is so concerned. This "volume energy," here the energy of the colloidal solution, is a function, first, of the number of molecules or of particles, and of their velocity, and, therefore, second, of the chemical splittings and combinings, and of the temperature, respectively.

With the temperature and volume constant, the decrease in volume energy demands a correlative decrease in the number of molecules, or of colloidal particles, or of both, as accompanying cleavage. This decrease would take place as a result in turn of a combining, to a definite degree of course, of molecules and of particles, which chemical change would be accompanied by the passing of energy from the system (ovum) to the environment in the form of heat. At least part of the "resultant" decrease in the volume energy of the system is to be accounted for in this way. Concerning the remainder of the decrease the evidence shows that its reappearance is in the form of the increase in the energy of the surface and in the mechanical energy or work done in the moving of the "mass" surrounding the system as environment. Under normal conditions it is with the intensity of the "surface pressure" equal to the opposed intensity from within that an equilibrium of form continues.

What now, finally, is the meaning of the fact that it has been possible to determine the energy of segmentation according to the method presented? That meaning I propose to summarize, for I believe it stands firm, even on the basis alone of the limited numerical results obtained.

As a first step in the demonstration it was necessary to state briefly the principles which it was my purpose to apply, etc. These were then formulated and shown to be epitomized in the fundamental equation

$$W + (U_2 - U_1) = I \frac{dW}{dI}$$

The "work integral" was then shown to be a special case of this



formula, with the result that the successful application of the former to segmentation would mean also the validity of the latter and therefore of the Four Laws for this event. To it there would apply, then, the principles of Determinism, Potential Difference, Conservation, etc.

But these laws are, seemingly, largely if not wholly quantitative, while on the other hand the organism, *e. g.*, the ovum, is qualitative as well as quantitative. What, then, is the relation of these Laws to the qualities, and what are these?

To answer the former question first, it may be said, that qualities in both the inorganic and the organic world are, at the same time that they are qualities, also quantities; and quantities are either extensive or intensive. Of qualities certain empirical laws are discoverable, while between *these* laws similarities are in turn found which lead to the Four Laws epitomized in equation [8]. Thus we get a "natural classification" of laws. From this it will be seen that the generic characteristics, so expressed, have the relation to that from which they are derived of being ultimately incorporate in the concrete qualities, and that they do not, although they are predominantly quantitative, simply exist alongside of these as a separate and distinct aspect. Rather, the Four Laws express the common quantitative aspects of these concrete qualitative-quantitative phenomena.

This view is directly opposed to that which regards the Four Laws, because quantitative, as "not touching" the concrete qualities, and then finds that these last, because not so "touched," furnish opportunity, especially in organisms, for Indeterminism, Regulation, Freedom, Entelechies, etc.

But what are the qualities themselves? Are they not of things, events and relations? Our answer is: Let "thing" be equated with system; then system implies parts, and these may be either atoms, or coexisting energies, or both. In either case *some* of the qualities of the "thing" result from the coöperation of the parts or elements, whose qualities are different from those of the whole which they form, the test being, that if isolated their qualities are found to be unlike those resultant ones; this bringing about by the parts of qualities which they themselves have not, may be called

“creative synthesis.” *Other* qualities of the system are the same as those which the parts retain when isolated. The latter give an additive result in the complex, the former do not.

It is now possible to make a statement as to what the cell is, and, if we may generalize, to answer our major question as to just how different organic phenomena are from inorganic.

According to our hypothesis the cell is a system, a complex of energies or of colloidal particles, etc. Some of these components can be isolated, and, with this done, are found to follow the usual inorganic laws; these they are therefore assumed to follow when in the complex. The same assumption is also made for those components which cannot be isolated; that is, the contrary position that such an “exclusion” demonstrates the presence of an irreducible, organic, vital remainder is held to be incorrect in view of the successful application of the energy-laws to the organism as a whole.

The qualities of the cell, are some of them, identical with the qualities of the parts and are the additive result of these, while others are the result of the “creative synthesis” of two or more constituent energies, etc. All these qualities are at the same time quantities, either extensive or intensive.

Now, without it being necessary to treat either these energies or the qualities of the system analytically, it has been possible, since at least some of them act together to produce, or are identical with, the event of cleavage, to measure this as a whole and bring it under the Four Laws. Thus are the subordinate events which contribute to this resultant event also brought into the range of the validity of these Laws.

The qualities of the organism—which are also quantities—are, accordingly, shown to be qualities which on this quantitative side have certain characteristics which are the same as those of the inorganic world—namely, those characteristics which the Four Laws formulate. Conversely, the Four Laws, as formulating these common characteristics, and as epitomized in equation [8], bring the concrete phenomena, both organic and inorganic, and the series of empirical laws into a “natural classification.”

But this does not do away with the fact that here in the so-called

organic realm, as in the inorganic, there are specific qualities which differentiate each class of complex from every other class, or, indeed, each individual from every other. The organism may have, therefore, qualities which, as such, are specifically different from any found in the inorganic realm; a "reduction" of these to inorganic being as impossible as is that of one inorganic quality to another. On the other hand, these very qualities, in that they are at the same time quantities, are like the inorganic in that they have in common with these the characteristics formulated in the Four Laws. *In just this respect there is no difference between organic and inorganic*; they are in the same realm whatever that be called. The only difference between organic and inorganic which still remains is, then, just that difference which persists between specific and specific, a difference which holds as good within the inorganic realm as it does between it and the organic. The only ground remaining for holding a distinction between the two realms is, that, taking the same level of classification or comparison, the differences between certain complexes, called inorganic, is less than the difference between these and certain others called organic. But even this does not do away with the necessity of bringing all into one realm in which the principles of Conservation, Potential, Determinism, etc., are valid. I conclude, then, that all events, both organic and inorganic, take place in full conformity with these principles, and that there is no ground for holding or interpreting organic events, etc., to furnish contradiction or evasion of them.

# FACTORS IN THE REGENERATION OF A COMPOUND HYDROID, EUDENDRIUM RAMOSUM<sup>1</sup>

BY

A. J. GOLDFARB

WITH TWO FIGURES

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## PRELIMINARY STATEMENT

Loeb's pioneer experiments on regeneration in hydroids, have stimulated a large number of investigators to study the effects of external and internal factors in these animals, especially upon unbranched or slightly branched forms like *Tubularia*. Most hydroids are affected by the same agencies, but not to the same degree; that while gravity is the determining condition in one hydroid, contact or regional differences or "polarity," determines

<sup>1</sup> I am deeply indebted to Prof. Thomas H. Morgan, who suggested these studies and who rendered much valuable advice and assistance to me throughout the course of these investigations. My thanks are due Prof. Edmund B. Wilson for the privilege of occupying the Columbia University Table at the Marine Laboratory at Wood's Hole, Mass., and to Prof. C. W. Hargitt for many valuable suggestions.

the kind of regeneration in other hydroids. In the following study of *Eudendrium ramosum*, I have attempted to examine nearly all the known factors, external and internal, that enter into the life of this hydroid, especially those that take part during growth and regeneration.

*Eudendrium ramosum* consists of one or more main stems, bearing pinnately arranged lateral branches which, in turn, branch again and again, finally ending in pedicels each bearing a polyp. When kept in an aquarium the polyps disappear and regenerate periodically. A few preliminary experiments made it clear that the method,<sup>2</sup> previously used, of adding the number of hydranths regenerated on a stem, each successive day after amputation of the polyps, did not give an accurate idea of the actual number of *different* hydranths regenerated in a given time. If, for example, one or more hydranths should regenerate on a branch at about the same time that an equal number of other hydranths degenerated, the records would not show the formation of new polyps. In the following experiments the exact number of different hydranths produced each day was recorded by the aid of daily diagrams of each stem and branch showing the presence or absence of polyps, buds and stolons.

Hydranths appear within two or three days after amputation. Later some or all of the regenerated hydranths may disappear to be replaced in part or in whole by new hydranths; or other cut ends, devoid of hydranths, may regenerate them now for the first time. In order to condense into the smallest space the data essential to an understanding of the phenomena, the number of hydranths that appear within three and six days respectively, after the removal of polyps, are quoted in the following tables, unless specifically mentioned to the contrary. When fractions are used the numerator represents the number of new hydranths formed in the time stated; the denominator indicates the number of lateral branches or pedicels removed. For convenience these fractions are usually reduced to per cent.

It is nearly impossible to obtain stems absolutely alike in all

<sup>2</sup>Light as a Factor in the Regeneration of Hydroids: Goldfarb, Journ. Exp. Zool., 1906.

respects. For practical purposes, stems that resemble each other in size, number and size of branches, that come from similar regions in the colony, and that are removed from their habitat at the same time, will be called "similar stems."

#### EFFECTS PRODUCED BY REMOVING LATERAL BRANCHES OR PEDICELS

*Experiment I.* This experiment was undertaken to determine whether stems, bearing lateral branches but with the pedicels and their polyps removed, would regenerate a greater or less per cent of polyps than stems with all the lateral branches trimmed off close to the main stem. On one side of a large stem the branches were cut off close to the main stem, while on the other side only the pedicels were removed. From a second stem the lateral branches were amputated on both sides; and from a third the polyps only were removed. The records for each stem were as follows:

TABLE I

No. of stems	Regenerated in			
	3 days		6 days	
	Pedicels only removed	Branches removed	Pedicels only removed	Branches removed
1.....	$\frac{2}{3\frac{1}{4}}$	*	$\frac{2}{3\frac{1}{4}}$	*
2.....		$\frac{3}{11}$		$\frac{7}{11}$
3, 4.....	$\frac{4}{18}$	$\frac{5}{17}$	$\frac{6}{18}$	$\frac{14}{17}$
5, 6.....	$\frac{2}{3\frac{1}{4}}$	$\frac{10}{17}$	$\frac{3}{3\frac{1}{4}}$	$\frac{2}{17}$
Total.....	24%	35%	42%	77%

\* The figures for each side of the stem given separately.

The conclusion is obvious, viz: that colonies from which all the branches have been removed regenerate more hydranths than those from which nothing but the pedicels and their hydranths were amputated. This conclusion was corroborated by later experiments.

All the lateral branches were removed from stems used in the succeeding experiments.

EFFECTS DUE TO REGIONAL DIFFERENCES<sup>3</sup>

*Experiment 2.* Is the tendency to regenerate polyps more strongly developed in one region of the stem than in another, or is the same average number produced in all regions of the same size? Pieces from a series of large stems were compared and the number of polyps produced in each was separately estimated, viz: (1) The basal end of a stem, about one-tenth of the whole stem, (2) the basal half of a second stem, (3) an entire third stem, (4) and the two halves of this stem separately considered.

TABLE 2

Number of hydranths regenerated on

	Entire stem	Distal half of entire stem	Basal half of entire stem	Basal half	Basal tenth
3 days.....	$\begin{cases} \frac{6}{20} \\ \frac{3}{8} \\ \frac{13}{24} \end{cases}$	$\begin{cases} \frac{6}{10} \\ \frac{3}{8} \\ \frac{12}{12} \end{cases}$	$\begin{cases} \frac{0}{10} \\ \frac{0}{8} \\ \frac{1}{12} \end{cases}$	$\begin{cases} \frac{5}{9} \\ \frac{0}{10} \\ \frac{0}{10} \end{cases}$	$\begin{cases} 0 \\ 0 \\ 0 \end{cases}$
Average, 36%		70%	3%	17%	0%
6 days.....	$\begin{cases} \frac{17}{20} \\ \frac{11}{16} \\ \frac{22}{24} \end{cases}$	$\begin{cases} \frac{11}{10} \\ \frac{9}{8} \\ \frac{17}{12} \end{cases}$	$\begin{cases} \frac{3}{10} \\ \frac{2}{8} \\ \frac{1}{12} \end{cases}$	$\begin{cases} \frac{6}{9} \\ \frac{4}{10} \\ \frac{3}{10} \end{cases}$	$\begin{cases} 0 \\ 0 \\ 0 \end{cases}$
Average, 83%		133%	33%	44%	0%

No regeneration occurred on the small basal pieces until the seventh day after amputation. Even then very few polyps appeared. The basal halves regenerated 17 per cent, the entire stems much more, namely, 36 per cent. More striking, however, is the difference in the regenerative power of the basal and distal halves of entire stems, for 70 per cent regenerate on distal halves, but 3 per cent on basal halves. The figures for six days reinforce these conclusions. Smaller stems, however, do not reveal this sharp contrast in the regeneration of the two halves of stems.

*Experiment 3.* Similar stems were cut into three nearly equal parts. The distal thirds regenerated two days after amputation; most of the middle pieces did not regenerate till the third day, and the basal pieces, not till the third or fourth day. The question of

<sup>3</sup>Some very interesting facts in this connection are given by Gast and Godlewski in *Die Regulationserscheinungen bei Pennaria cavolinii*, Archiv f. Ent., Bd. 16, 1903.

rate of development will be discussed later. For the present it will suffice to state that because of this difference in the rate of development, the latent period<sup>4</sup> was not computed and the number of complete polyps produced two days after their first appearance (which may be the fifth or sixth day after amputation), and the number produced within the next three days, were recorded.

TABLE 3

Regenerated on																Average	
	2 days							5 days								2 days	5 days
Distal thirds	2	6	7	5	4	9	7	4	8	9	9	5	12	10	12	66%	100%
	6	7	9	8	7	9	11	6	7	9	8	7	9	11	9	7	8
Middle thirds	4	6	7	5	6	6	5	7	10	8	5	12	12	7	8	61%	101%
	7	8	9	8	9	11	4	7	8	9	6	12	9	11	9	8	8
Basal thirds	2	6	3	1	4	3	3	3	7	5	3	7	10	7	7	42%	77%
	7	9	9	8	9	10	11	7	9	9	8	9	10	11	9	6	8

We may conclude that the distal and middle pieces regenerate practically the same number of hydranths but far in excess of the basal pieces.

*Experiment 4.* The last experiment was modified to the extent of using not the main stem but the *lateral branches* from the distal, middle and basal regions of the stem. The distal branches were small and delicate, quite different from the middle and basal branches which owing to the smaller stems used, resembled each other closely.

TABLE 4

Regeneration on	2 days	5 days
Branches from distal region . . . . .	37%	57%
Branches from middle region . . . . .	50%	79%
Branches from basal region . . . . .	68%	104%

Regeneration of lateral branches differs at different levels. It is greatest on branches taken from the basal regions (of small stems) least on branches taken from distal regions. An explanation of these phenomena will be attempted under the caption of "cœnosarc."

From the evidence already cited we may summarize as follows: *The distal half of a stem regenerates a much larger number of polyps than the corresponding basal half. On the contrary, branches*

<sup>4</sup>See Rate of Regeneration.



from the apical region give rise to fewer polyps than the branches from the middle and basal regions. While polyps at the apical end of stems are common, they are rare on the apical ends of branches.<sup>5</sup>

#### HETEROMORPHOSIS

The phenomenon of heteromorphosis<sup>6</sup> in hydroids, has been investigated by Driesch, Loeb, Morgan, Stevens and others, particularly upon unbranched or slightly branched colonies. It was hoped that experiments upon a much branched hydroid like *Eudendrium ramosum*, would afford further insight concerning:

- 1 The conditions underlying the formation of heteromorphic polyps.
- 2 The effects of such polyps upon the regeneration of other polyps on the stems.
- 3 The rate of development at different levels of the stem.
- 4 The coenosarc, its movements and its effects in the production of heteromorphic polyps.

A great many observations on large stems of all kinds had shown that polyps are rarely produced at the basal region, particularly at the basal cut ends, though common enough at middle or distal regions. Smaller stems or pieces of large stems—bearing from 15 to 20 branches—regenerated more basal hydranths than the much larger stems, though still less than the number of apical polyps.

*Experiment 5.* Large stems with lateral branches removed were cut into three nearly equal pieces. Further details are given in Experiment 3. The number of hydranths regenerated at the oral and the basal cut ends of each piece, two and five days after their first appearance, are given in the following table:

<sup>5</sup>See Gast and Godlewski, loc. cit.

<sup>6</sup>The following papers on Heteromorphosis and Polarity give various hypotheses to account for these phenomena in hydroids: Bickford '94 J. Morph.; Driesch '92 Biol. Cent., '96 Vierteljahrs-schr. Nat. Ges. Zurich, '97 Archiv f. Ent., '99 Archiv f. Ent.; Loeb '91, Ueber Heteromorphose, Würzburg; Morgan '01 Biol. Bull., '01 Archiv f. Ent., '04, '05, '06 Journ. Exp. Zool.; Morgan and Stevens '04 Journ. Exp. Zool.; Stevens '02 Archiv f. Ent.

TABLE 5

Polyps regenerated on		2 days	5 days
Distal third of stem	{ oral ends .....	60%	60%
	{ basal ends .....	30%	100%
Middle third of stem	{ oral ends .....	80%	100%
	{ basal ends .....	40%	70%
Basal third of stem	{ oral ends .....	70%	100%
	{ basal ends .....	20%	30%

With distal pieces excepted, the branchless parts of a *stem* regenerate decidedly more hydranths at the apical end than at the opposite or basal end.

*Experiment 6.* The above results contrast sharply with those in this experiment in which only *lateral branches* from different regions of the stem were used.

TABLE 6

Polyps regenerated on		2 days	5 days
Branches from distal part of stem	{ oral ends .....	12%	37%
	{ basal ends .....	37%	50%
Branches from middle part of stem	{ oral ends .....	12%	12%
	{ basal ends .....	62%	112%
Branches from basal part of stem	{ oral ends .....	25%	37%
	{ basal ends .....	37%	87%

The lateral branches from any level of a stem produce a far greater number of heteromorphic than apical polyps.<sup>7</sup> Under the caption "cœnosarc" this will be explained.

*Experiment 7.* In Experiment 6 all secondary branches were removed. In the following experiment these were not removed nor were the polyps amputated. The regeneration at the basal and apical ends only was recorded. While the polyps were disintegrating, stolons were forming at all the basal cut ends, fastening the pieces to the bottom or sides of the dish. Later stolons were present on several pieces at both oral and basal ends. These stolons often grew to a remarkable length as long as or longer than the original specimen. Usually they gave rise to several branching stolons which regenerated one or more polyps, even as

<sup>7</sup>Driesch, Morgan and Stevens found that the apical ends of pieces taken from any region were first to regenerate and produced a greater number of polyps than the basal ends.

many as nine polyps were present at the basal end of one piece. These hydranths did not appear before the third or even the fourth day after removal of the branches. The following table gives some of the details of the experiment.

TABLE 7

Polyps regenerated at basal end of	3 days	6 days	10 days	Polyps regener- ated at oral ends in 10 days	No. of branches that regenerated basal polyps
Distal branches .....	0	80%	160%	0%	90%
Middle branches .....	0	0%	100%	10%	70%
Basal branches .....	0	50%	120%	20%	50%

The presence of polyps on the branch retards regeneration but does not prevent the formation of polyps at the basal cut ends. The figures, particularly in the last column, of the above table seem to indicate a maximum tendency toward the production of heteromorphic polyps on distal branches, less and less on middle and basal branches, respectively.

## SUMMARY

*The mid and basal thirds of a stem behave quite differently from the distal third and from the lateral branches, in so far as the relative number of hydranths regenerated at the oral and basal ends is concerned. The distal region of stems and the lateral branches from any region produce a greater number of heteromorphic and fewer apical polyps, than do the median and basal thirds of the stem. Lateral branches from which polyps had not been removed likewise produce more polyps at the basal than apical ends.*








*Experiment 8.* In the preceding experiments the presence of lateral free cut ends may have introduced disturbing factors not yet fully considered. In order to avoid these influences, some or all of the lateral cut ends were ligated on a series of similar stems as follows:

- a* the apical end only;
- b* the apical end and the two lateral ends on distal half of stem;
- c* the apical end and all the lateral ends;
- d* all the lateral ends;

- e* all the lateral ends and the middle of the stem;  
*f* the middle of the stem only;  
*g* all the lateral ends, then stem was cut into two equal parts;  
*h* not ligated—control stems.

The rate of development in the different series varied considerably. In the table the number regenerated for two and five days after the first appearance of polyps is given.

TABLE 8

								
Polyps reg.	on							
in								
2 days ...	Oral ends .....	82%	66%	87%	88%			
	Lateral ends .....	54%		25%		52%	44%	
	Basal ends .....	61%	75%	75%	100%	88%	100%	100%
	Total Average...	66%	70%	62%	94%	70%	72%	100%
5 days ...	Oral ends .....	123%	116	125	155			
	Lateral ends .....	94%		100		66	76	
	Basal ends .....	102%	123	100	155	122	137	118
	Average .....	106%	124	108	155	94	106	118

It will be observed, firstly, that hydranths are normally regenerated more readily at the oral rather than the basal ends of stems. Secondly, with the same number of ligatures to the stem, the number of polyps regenerated depends on the location of the ligated branch or branches; that is to say, tying a lateral end near the basal region of the stem is less efficacious in stimulating basal regeneration than a ligature on a lateral branch nearer the distal end, which in turn is less efficient than a ligature at the apical end. Thirdly, the total number of polyps produced or at least the number of basal polyps, is in a general way proportional to the number of ends ligated; the more ends ligated the greater the total and basal regeneration. It should be recalled, however, that ligating four lateral branches is less effective than one apical ligature. Fourth, a ligature around the middle of a stem increases the number produced at both oral and basal ends but not to the

same degree, causing a slight increase at the oral end, and a considerable increase at the basal end. Fifth, the influence that a ligature exerts, does not, as a rule, affect the next lateral free end to the same degree as the axial free end, particularly the basal free end, even though such axial end is not nearest to the ligated branch. Sixth, while the figures in the table are not absolute they show in a general way which arrangement and number of ligatures are more effective in bringing about an increased basal regeneration.

*Experiment 9.* We may now turn our attention to heteromorphosis shown by very small pieces of stems and branches. The apical ends of a series of stems, including the distal two pedicels and their polyps, were removed. The apical polyp was then cut off. In one lot the lateral branch was ligated, in the second, they were not ligated; in both the axial ends were equidistant from the lateral pedicel. Polyps appeared at one or both ends either simultaneously, or more frequently the basal end appeared one day before the oral hydranth. Sometimes stolons were produced at one or both ends. *The basal ends regenerated more rapidly and in greater numbers than the oral ends of these pieces.* The actual figures are as follows:

TABLE 9

Polyps regenerated on	2 days	3 days	4 days
Oral ends .....	0	17%	25%
Basal ends .....	7	25%	45%

The same results obtain when similar pieces taken from different regions of the stem are used. About three times as many polyps are produced at the basal as at the oral ends in six days. These facts indicate that very *small pieces of stems, regardless of the region from which such pieces are taken, tend to regenerate a far greater number of heteromorphic polyps and to produce these faster than oral polyps.*

The following three experiments give further evidence concerning heteromorphic regeneration on small pieces from similar or different levels of stems.

*Experiment 10.* The apical ends of a number of stems and branches were cut beyond the distal node. These pieces about

1½ mm. long and bearing a distal polyp, were kept carefully oriented. The third day after amputation the apical hydranths had disintegrated. On the fourth day polyps were first regenerated and appeared invariably at the basal ends. One lateral polyp only was produced, two oral polyps appeared on the seventh day, therefore not mentioned in the table; in no case were polyps regenerated at both ends. The following table gives the actual figures:

TABLE 10

Polyps Regenerated on	3 days	6 days
Oral ends.....	o	o
Lateral ends.....	o	3%
Basal ends.....	o	44%

When pieces of the same kind as the preceding were ligatured at their distal ends, regeneration was not accelerated, nor was the number of basal polyps increased. The figures are remarkably like those in the preceding, viz:

TABLE 11

Polyps regenerated on	3 days	6 days
Oral ends.....	o	7%*
Basal ends.....	o	43%

\*The coenosarc withdrew completely out of perisarc and then formed an oral hydranth. This matter will be taken up under "coenosarc."

*When pieces about 1½ mm. long (smaller than those in Experiment 9) were used, the number of heteromorphic polyps was practically the same as in the experiment just mentioned, though in proportion to the number of oral polyps there was a far greater basal regeneration in the smaller pieces. Furthermore, an apical ligature did not accelerate regeneration nor did it increase the number of basal polyps.*

*Experiment II.* This tendency to produce more polyps at the basal ends is also clearly demonstrated with internodes of stems. The internodes of several stems were cut off and oriented, and so arranged that it was possible at a glance to tell to which part of the stem each internode belonged. All doubtful pieces were rejected. The 55 internodes regenerated as follows:

TABLE 12

Polyps regenerated on	3 days	6 days
Oral ends .....	9%	39%
Basal ends .....	16%	52%

In these pieces, each about 1 mm. long, more basal polyps are produced. A considerable number of internodes formed hydranths at each end, in some cases simultaneously. This fact indicates that some of these very short pieces had the potency to produce more than one hydranth. The majority regenerated but a single polyp. Regional differences were not apparent in this experiment. It cannot be said, for example, that regeneration was retarded or accelerated, increased or decreased in one region more than in another; nor was the formation of basal hydranths peculiar to any one region or level.

From these experiments we conclude that in the *small pieces mentioned, a greater number of heteromorphic than apical polyps are produced; that this increase is not associated with any particular level of the stem; that oral polyps are rarely formed on distal pieces, though many regenerate on pieces from middle and basal regions of the stem.*

*Experiment 12.* To determine whether rapid growth first in one direction then in the opposite direction, could be effected, the new basal stems from a number of pieces were removed, viz: *B* pieces of the diagram. There developed from the basal end *b*, of these basal stems many hydranths which grew with remarkable celerity in the original direction toward *a*; there also regenerated actively polyps at the oral (*c*) and the lateral ends of the *B* pieces. There was no reason to doubt that the removal of the *C* pieces would result in the formation of hydranths at the new basal end of *C*. If lateral branches instead of basal branches be repeatedly removed the results are essentially the same. The actual figures are given below:

TABLE 13

Polyps regenerated on basal pieces of <i>B</i>	3 days	6 days	9 days
Oral ends .....	0%	26%	53%
Basal ends .....	33%	40%	53%
Polyps regenerated on basal ends of lateral branches			
Oral ends .....	6%	36%	46%
Basal ends .....	0%	23%	50%

The above tables give the number regenerated at the basal ends of pieces 3, 6 and 9 days after amputation. The oral (and lateral polyps also) had not been cut off but disappeared within two days. Regeneration, therefore, at oral and lateral ends could not take place till one or two days after the basal polyps appeared.

*Regeneration in one direction, viz: from the basal end of B piece does not inhibit regeneration in the opposite direction at c; rapid growth and differentiation take place synchronously in opposite*

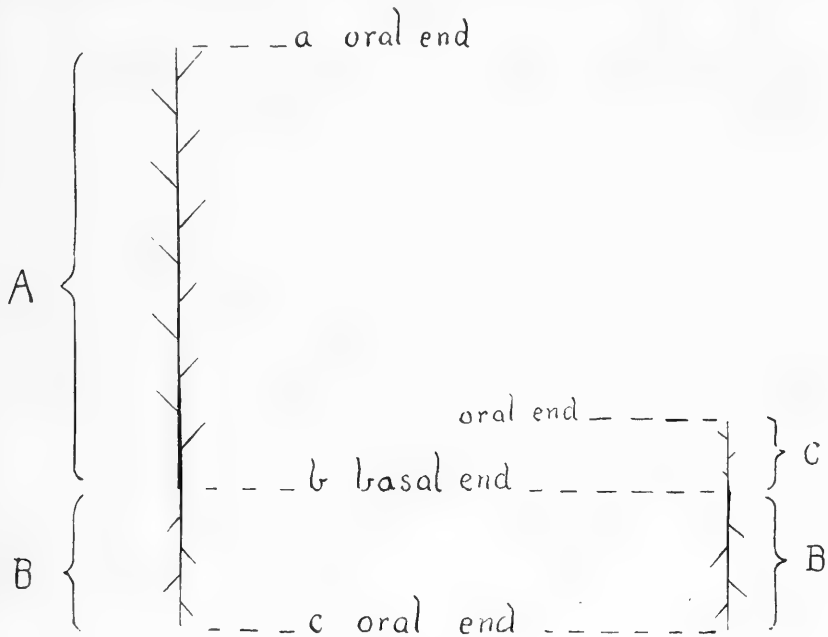


Fig. 1

*directions.* This would lend weight to the view that regeneration is not dependent on internal changes of the entire stem or even parts of the stem but is rather due to reactions at the cut end only.<sup>8</sup>

An examination of Tables 14 and 15 will make it clear at a glance that gravity is a considerable factor in determining the increase or decrease of the number of hydranths at the upper ends (toward the zenith), regardless whether such ends be oral or basal. Erect

<sup>8</sup> Except for minute pieces, see also Experiment 25 on this point.



stems regenerate in 3 days, 50 per cent at the oral and 45 per cent at basal ends. Inverted stems—with basal ends pointing toward the zenith—in the same time, regenerates 35 per cent at the oral and 60 per cent at the basal ends. The figures for 6 days emphasize the point, viz: Erect stems regenerate 50 per cent at the oral, 80 per cent at basal ends, inverted stems 35 per cent at oral and 115 per cent at basal ends.

Injury to different parts of a stem does not affect the regeneration at the axial ends of the stem. Nor does increasingly diluted or concentrated sea-water influence the number of hydranths at basal or oral ends. Heteromorphosis occurs independently of these influences.

#### COENOSARC<sup>9</sup>

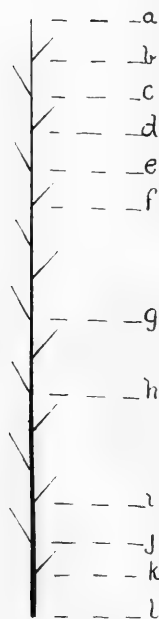


Fig. 2

The cœnosarc may readily be observed in the more distal parts of a colony, for the perisarc there is thin, almost transparent and seldom covered with débris, polyzoans or hydroids, commonly found on the more basal regions. The cœnosarc is composed of a hollow cylinder surrounded by the perisarc. The lumen, within the cœnosarc is circular in cross section and differs from *Tubularia*<sup>10</sup> in which the lumen is divided into two almost separate compartments by a central partition along the whole length of the stem.

It has already been pointed out that the number of hydranths regenerated at the oral or at the basal ends, depends upon whether lateral branches or stems were used, and if the latter, upon the region from which the piece was taken. *Previous experiments and direct observations prove conclusively that the cœnosarc withdraws from the distal*

<sup>9</sup>Gast and Godlewski's account of the cœnosarc in *Pennaria cavolinii* is extremely interesting in this connection. Hargitt, G. T., '03 gives an account of the histologic structure of the cœnosarc. *Archiv für Ent.*, Bd. 17.

<sup>10</sup>Stevens, N. M., '02 records movements of the cœnosarc in *Archiv f. Ent.*, Bd. 15. Stevens '01, '02, *Archiv f. Ent.*, Bd. 13 and 15.

ends of all branches, cut at any level, and from the distal ends of pieces from the apical region of the stem. The *cœnosarc* also withdraws, as a rule, from the distal ends of small pieces from any part of the colony. In the diagram of a large stem *a* represents the oral, *l* the basal end. We can foretell with a fair degree of accuracy whether polyps will be formed at one or the other ends of pieces and the relative number regenerated at each end, provided we know the level at which the cuts were made. In pieces *b c*, no hydranths will appear at *b*, many at *c*; in pieces *f h* or *g i* more hydranths will appear at *f* and *g*, respectively, than at *h* and *i*. In region *a f* the nearer to *a* the oral end of a piece lies the fewer the oral polyps; in region *j l* the nearer to *l* the basal end of a piece lies the fewer the basal hydranths regenerated.

The recession of the *cœnosarc* from the distal end may extend only one internode or half a dozen or more, and the hollow perisarc thus produced often cracks and breaks off.<sup>11</sup> In small pieces the *cœnosarc* may move not only toward the basal end but through the basal end entirely free from the perisarc, leaving the empty perisarc behind. Placing inverted stems in sand accelerates the basal movement of the *cœnosarc*, so that the parts embedded in sand become entirely empty; the *cœnosarc* is found only in the basal regions surrounded by water. In erect stems under the same conditions the *cœnosarc* withdraws somewhat from the distal end while at the basal end it either (1) does not withdraw at all, in the majority of cases, and in spite of the adverse conditions, (2) or slightly withdraws, (3) or disintegrates, the result of the ravages of large numbers of ciliate protozoa.

Two counteracting tendencies may be said to be present at every cut end of a stem or branch, the resultant of which determines whether a hydranth will or will not be regenerated, and whether regeneration will or will not be retarded; first, the movements of the *cœnosarc* from the distal cut ends already described, second—and I believe second in point of time—the regeneration of new tissue, which is negatively geotropic and,

<sup>11</sup>Gast and Godlewski, loc. cit.

therefore, tends to grow upward. The more cœnosarc present near the cut end, as determined by its width and density, the greater the regeneration. The less impeded, the greater the movement of the cœnosarc. Whether enough cœnosarc is present or is regenerated, to make up for the recession of the cœnosarc will determine whether or no hydranths will appear at the cut end. The basal movement brings additional cœnosarc to the basal end, condenses it greatly and thereby increases the number of basal polyps, on small pieces, on lateral branches and on pieces from the distal region of stems. *The cœnosarc in the middle and basal parts of the stem* does not withdraw from the oral cut end and, as a matter of fact, there are more hydranths at the oral than basal ends. In this hydroid at least, we do not need to call to our aid "formative stuffs,"<sup>12</sup> and other hypothetical internal forces and materials to account for heteromorphosis, for the movements of the cœnosarc account for the presence or absence of polyps at some levels and not at others.

The cœnosarc of stems kept long in the aquarium often becomes fragmented. Fragmentation is the result of a splitting of the cœnosarc at one or another of the internodes; the cœnosarc thins rapidly at these points, and finally breaks into pieces entirely independent of each other. Each part may move into the nearest lateral branch and give rise to polyps, or it may remain in the stem. Not infrequently in the latter case it contracts at both ends ultimately forming an ellipsoidal dense mass of cœnosarc near the basal end of the stem. It is believed that these hydroids winter over in this contracted condition. When stems are subjected for a long time to adverse conditions the cœnosarc forms this dense ellipsoidal mass. When such stems, which could no longer be made to regenerate, were cut into smaller pieces, polyps regenerated provided the cœnosarc was injured.

With a low power of the microscope the lumen within the cœnosarc is seen to be filled with a colorless fluid in which myriads of colorless granules float. These move slowly toward one end of

<sup>12</sup>The hypothesis of specific stuffs, moving in definite directions, was developed by Bonnet, later by Sachs, and still further perfected by Loeb. For criticism, see Morgan '01 *Archiv f. Ent.*, Bd. 11, '02 *ibid.*, '04 *Journ. Exp. Zool.*

the stem; in pieces  $1\frac{1}{2}$  inch long the trip from one to the opposite end takes from  $1\frac{1}{2}$  to 3 minutes. As the granules accumulate at this end the stream moves more and more slowly until it finally ceases. There may be a respite of a few minutes and then the stream courses in the opposite direction at first slowly then faster and faster, and finally slowly again as the granules pile up at the other end. The performance is repeated over and over again; the time for each trip may vary considerably. Now and then groups of granules are violently whisked about or "tremble," the result of ciliary movement of the endoderm. Granules were never seen to pass out of the prostomium of the living polyp. After disintegration of the polyps dark red masses<sup>13</sup> were frequently observed marking the spot where the polyps had been.

The current in a lateral branch may be continuous with that in the main stem, moving at the same rate and in the same direction, or it may be independent, and even contrary to the stream in the stem. The central stream may be continuous or divided into two or more independent streams. Though the *cœnosarc* in branches and stem is continuous, the streams within the *cœnosarc* of these parts may behave independently. The continuity of the *cœnosarc* may be permanently broken by pressing a stem firmly with the side of a needle. The *cœnosarc* is cut into two parts which separate more and more from each other. If gently pressed, a dent is temporarily produced in the *cœnosarc* which slowly recovers its normal shape.

The number of hydranths that arise from the basal cut end of pieces is closely associated with the amount of *cœnosarc* near such ends. Other conditions being equal the more *cœnosarc* at or near a cut end the more hydranths produced. Several conditions must, however, be taken into account. A large stem does not necessarily contain more *cœnosarc* per unit of length than another one-half as long. Much depends on the more or less contracted condition of the *cœnosarc*. If it be attenuated, as in rapidly growing branches, it will have per unit of length less regenerative potency than the more concentrated *cœnosarc* usually

<sup>13</sup>These dark red bodies are probably analogous with the red bodies resulting from the metabolic changes in *Tubularia*, studied by Bickford '94; Stevens '01 and '02, and Loeb '91, and Morgan loc. cit.

found near the basal region of stems. Careful measurements of the diameter of the cœnosarc at different levels of large stems actually shows that normally the diameter of the cœnosarc decreases toward the distal end and conversely increases basally. The relation between size and the number of hydranths regenerated, particularly at the basal end is shown by the following observation. Pieces less than 1 mm. long never produced a complete polyp, though they often regenerated shoots at one or both ends. Pieces as small as two-fifths mm. long developed shoots at one end. Larger pieces 1 to 2 mm. long may regenerate one polyp at each end though usually at the basal end only. Still larger pieces regenerated two or three polyps from one basal end, whereas larger median or basal pieces produced as many as nine polyps from a single basal end.

#### STOLON FORMATION

Little has been said concerning stolon formation, partly because of its comparative rarity, partly because stolons are often with difficulty distinguished from branches. A stolon,<sup>14</sup> root, or hydro-rhiza is an outgrowth, positively geotropic and stereotropic in its reaction, which, when young, fastens itself by a sticky secretion to solid objects, and which does not directly give rise to polyps. In nature the stolon or stolon system anchors the hydroid. Less frequently, stolons may join two stems and sometimes the cœnosarc of stolon and stem may fuse.<sup>15</sup> In the laboratory, stolons may appear at any of the cut ends. Though most frequently observed at the basal, they may appear at any lateral or even oral end, singly or in groups of branching stolons. They may appear simultaneously at the oral and basal ends, or at one end only. The stolon may sometimes grow to a great length; in one instance a stem 27 mm. long regenerated a basal stolon 40 mm. long. Stolons may give rise to lateral branches usually pinnately arranged, which like pedicels end in hydranths. Some unbranched stolons after a time bend at their very ends and regen-

<sup>14</sup>The production of stolons has been shown in some species to be determined by gravity (Loeb '91), in others by regional peculiarities (Stevens '02), contact (Loeb), exhaustion (Driesch), by the kind of regeneration at the opposite end of piece (Morgan '01).

<sup>15</sup>See Stevens '02.

erate a polyp at the tip. In these, it is impossible to tell where the stolon ends and where branch begins. It is nearly always difficult to tell in advance whether a large growing shoot will ultimately become a branch or a stolon. Stolons in my experiments never regenerated stolons; when cut, only hydranths were produced at the cut ends, irrespective of the level at which the cuts were made.

When stems are subjected to adverse conditions, the cut ends may regenerate new tissue, which becomes surrounded by a sticky perisarc, which does not differentiate into perfect hydranths. The new tissue is really a modified stem, which under these adverse conditions may increase in length and is then called a "stolon;" when it is amputated or when it grows into a favorable environment the distal end frequently differentiates into a hydranth. When a large number of stems were placed in a shallow dish of water containing much *débris*, and the water was left undisturbed for many days, very few polyps appeared, while a remarkably large number of stolons were produced. When the water was frequently changed, however, the "stolons" invariably bore polyps. As the colony grows older, the perisarc of the stolons becomes thicker, less plastic and encrusted with *débris*. If the conditions remain constant, the "stolon" functions permanently as an anchoring organ and can no longer of itself produce polyps, though it has the ability to do so, if cut and removed to a favorable environment. "Stolons" are not limited to any particular region nor is their formation influenced by gravity, size of the piece, kind of regeneration at opposite end, etc. Their presence or absence, in this hydroid at least, is not an indication of the presence or absence of certain internal changes and is, therefore, useless as an index of the polarity of the stem.

#### RATE OF REGENERATION<sup>16</sup>

The term rate is here used to designate the interval between amputation and that point in the differentiation of the regenerating

<sup>16</sup>Loeb ('91) determined rate of development and rate of growth for different hydroids. Morgan and Stevens '04, *Journ. Exp. Zool.*, made careful observations on the rate of development at basal and oral ends of pieces.

tissues at which the tentacles of the new hydranth are clearly discerned. Under normal conditions two days is required. Under adverse conditions regeneration may not take place for four, five, six or more days. Regeneration does not extend over this entire period, at least in so far as visible changes are concerned. There is a latent period, during which no visible changes obtain and which normally covers but a few hours, but which under unwholesome conditions may extend over several days. Once regeneration begins development proceeds normally in about 12 hours. So that adverse conditions increase not the actual period of regeneration but this latent period.

In large stems the younger (distal) portions always regenerate polyps at least one day before the older (basal) parts. When stems were cut into three equal parts, Experiment 3, new polyps appeared on the different pieces in the following order:

TABLE 14

Polyps regenerated on	2d day	3d day	4th day
Distal thirds .....	8 polyps	1 additional polyp	1 additional polyp
Middle thirds .....	0	9 polyps	1 additional polyp
Basal thirds .....	0	6 polyps	4 additional polyps

*The distal thirds regenerated polyps earlier than the middle pieces which in turn regenerated polyps before the basal thirds.*

To determine whether the two cut ends at any level of a stem regenerate at the same time, the above pieces of stems were used.

*A, B, C* represents the distal, middle and basal pieces, respectively. At the cut *b c*, *b* is the basal end of the distal piece, and *c* the distal end of the basal piece. It was found that the *b* ends on *A* pieces regenerated on the average in practically the same time as the *c* ends on *B* pieces, and similarly for the *b* and *c* ends on the *B* and *C* pieces. It was found that the *b* ends regenerate first, just as often as the *c* ends and as often as the *b* and *c* ends regenerated simultaneously.

Although the distal region of a large stem regenerates polyps within two days or at least one day before the basal region, yet small pieces cut from the distal region (Experiment 10) never produce polyps before the third day and often not till the fourth day.

This retardation occurs irrespective of the region of the stem from which the pieces were taken, and is due solely to the small size of the pieces.

Medium size pieces (with 10 to 15 lateral branches) regenerate polyps at all the cut ends, including oral and basal ends, in approximately the same time. Ligating the distal end or ends accelerates basal regeneration. Ligating the distal end of small pieces taken from the distal region of a stem, does not however accelerate basal regeneration, because the *cœnosarc* withdraws from the distal end and the ligature does not affect it. When the single lateral branch of small pieces (Experiment 9) was ligated the rate of development was uninfluenced. Regeneration on small old (basal) pieces taken from large stems, is very slow; it may take six or seven days before polyps appear.

Embedding inverted stems in sand and to a less degree suspending them, stimulates the early formation of heteromorphic polyps, but the rate of development at other cut ends was not at all or but slightly affected. Lack of oxygen or low temperature or contact with a solid body or greatly diluted or slightly concentrated seawater retards the development of the first formed polyps. In the meantime the stems become more or less acclimatized to the new conditions and polyps thereafter are regenerated at a normal rate. Injuries of various kinds, such as lacerating and slitting the stems in many places or disintegration of *cœnosarc* in some of the lateral branches, does not retard regeneration at the other cut ends. All efforts to accelerate the normal period of regeneration in less than two days, failed.

#### EFFECTS OF GRAVITY<sup>17</sup>

*Experiment 13.* A series of stems were suspended vertically in a dish of water, some with the distal ends pointing upward (toward the zenith), the "erect" stems; others in the contrary direction, the "inverted" stems. The controls were placed hori-

<sup>17</sup>Loeb ('91) believed that gravity and light were the external factors that determined the kind and direction of growth. Also see Driesch ('99).



zontally on the bottom of the dish. The rate of development was practically the same in the three groups.

TABLE 15

on	Polyps regenerated in 3 days				Regenerated in 6 days			
	Oral	Lateral	Aboral	Total	Oral	Lateral	Aboral	Total
Erect Stems . . . . .	50%	54%	45%	53%	50%	86%	80%	83%
Inverted Stems . . . . .	35%	43%	60%	44%	35%	75%	115%	75%
Control Stems . . . . .	33%	37%	44%	37%	33%	48%	66%	48%
(In Contact)								
No. of branches used . . . . .								574

An analysis of these figures gives some interesting details. In the first place, erect stems regenerate a greater total of polyps than the corresponding inverted stems. Secondly, erect stems produce a greater number of oral and a greater number of lateral polyps than do the inverted stems. The lateral branches of inverted stems that bear polyps bend upward toward the basal end of the stem, and these branches are invariably longer than on the erect stems. Thirdly, by far the greatest number of basal polyps are produced on inverted stems, from which they grow upward, and directly opposite to the rest of the stem. If the basal branch of erect stems are long they too bend upward. No emphasis is laid on the control stems in this experiment, as they were influenced by contact with the dish, a disturbing factor at the time not fully appreciated.

*Experiment 14.* Fine sand thoroughly cleaned was put in a dish of water. Erect and inverted stems were embedded in the sand to varying depths. The controls rested horizontally on the sand. The diagrams in the table show the position of the stems; the horizontal lines represent the level of the sand, the parts below it represent the parts embedded in sand, the parts above the line, surrounded by water.

The conclusions from the previous experiment were more than corroborated. Regeneration on inverted lateral branches is largely inhibited. This is strikingly illustrated, on comparison of columns 2 and 4 and more particularly columns 3 and 5, in which the same number of lateral branches are free, but erect in the one series, inverted in the other. The erect stems of series 4

regenerates 33 per cent, the corresponding inverted stems of series 2 regenerates 8 per cent. Similarly, series 5 produces 36 per cent, the inverted ones 7 per cent. The branches in the two sets are too close together to warrant the belief that the difference in position on the stems can account for the large difference in regeneration. When inverted lateral branches do regenerate polyps, they invariably turn upward.

Embedding stems in sand affects regeneration just as a ligature tied around the stem at the level of the sand would do, for in both

TABLE 16

Polyps reg.	on						
in		%	%	%	0%	42%	8%
3 days ...	Oral ends .....		8	7	33	36	13
	Lateral ends .....						
	Aboral ends .....	33	58	66	—	—	25
	Total .....	33	24	18	28	37	14
6 days ...	Oral ends .....				50	58	33
	Lateral ends .....		38	21	41	55	28
	Aboral ends .....	74	140	158	—	—	58
	Total .....	74	73	48	44	56	35
	Total number of stems .....						72
	Total number of branches .....						281

cases regeneration at the free axial end (oral or basal) of the stem is stimulated. The number regenerated at the upper ends of these stems depends on whether erect or inverted stems were used. Regeneration at the basal ends of inverted stems is very much greater than that at lateral or oral ends, for example, 58 per cent are produced at the basal ends, 8 per cent at the lateral ends, 0 per cent at oral ends, in one series. Again 66 per cent regenerated at basal ends, 7 per cent at lateral and 42 per cent at oral ends of a second series. Furthermore, the total number of polyps produced on a stem

depends on how much of it is embedded in sand. The deeper the inverted stems are embedded, the proportionally larger total number is regenerated and vice versa. On the contrary, the deeper erect stems are embedded the proportionally fewer polyps produced.

*Experiment 15.* These conclusions were corroborated and extended by the following experiment in which the sand was banked at an angle of  $45^\circ$  to the horizontal.

TABLE 17

Polyps reg. in	on	1	2	3	4	5	6		
3 days ...	Oral ends . . . . .	25%	80	25%	75	50%	0	66%	50%
	Lateral ends . . . .	54	50	75	50	100	66	56	
	Aboral ends . . . . .	—	50	100	50	100	—	83	
	Total . . . . .	50	83	73	70	50	66	57	
6 days ...	Oral ends . . . . .	50	—	50	75	—	66	83	
	Lateral ends . . . .	79	96	94	122	0	83	92	
	Aboral ends . . . . .	—	50	150	100	100	—	116	
	Total . . . . .	75	90	95	116	50	77	93	

## SUMMARY.

*Hydranth bearing branches turn toward the zenith whatever the position of the stem, whether erect, inverted or inclined. Erect stems embedded in sand regenerate the largest total number of polyps, inclined stems less and inverted the least number. Regeneration at the oral ends and lateral ends of similar inverted stems is largely inhibited, but at the basal ends is remarkably stimulated.* It will be recalled that the cœnosarc withdraws from the distal cut ends of erect stems; but a ligature or its equivalent embedding in sand, causes the cœnosarc to move upward and regenerate an oral polyp. On inverted stems the cœnosarc under these influences, and particularly under the influence of gravity, withdraws not only from the distal end but from nearly all the lateral ends, toward the upturned basal end, resulting in an immense

basal and almost *nil* lateral regeneration. Furthermore, the total number produced varies with the amount of free cœnosarc (not embedded in sand).

EFFECTS OF CONTACT<sup>18</sup>

*Experiment 16.* Stems were suspended on two horizontal threads, others were in contact with the bottom of the dish. Hydranths appeared on the two series at the same time.

TABLE 18

Polyps regenerated on	3 days	6 days	No of branches
Suspended stems .....	46%	68%	248
Stems on bottom of dish .....	41%	48%	111

This experiment agrees with No. 13, in that stems surrounded by water and otherwise under identical conditions produce a larger number of polyps than those in contact with a solid body.

*Experiment 17.* Shallow V-shaped grooves were made in a cake of paraffin of such a width that when stems were placed therein the lateral branches were in contact with the sides or bottom of the grooves. A record of each lateral end was made, whether (*a*) it pointed upward and out of the groove and therefore not in contact with the paraffin, (*b*) it extended sideways and touched the sides of the groove, (*c*) it pointed downward, and therefore in contact with sides or bottom of the groove. In six days only 23 per cent of all the ends regenerated polyps. Further details will more clearly illustrate to what degree contact suppresses hydranth formation. During the first six days but 7 per cent of all downward pointing branches (in contact), 8 per cent of all sideways pointing branches (in contact), 43 per cent of all upward pointing branches (free) regenerated polyps. Two hundred and thirty-six branches were used in this experiment.

*Experiment 18.* Stems were put within glass tubes, 1 to 1½ mm. inside diameter. One or both apices of some stems extended beyond the tube, in others several branches protruded beyond the tube, or short glass rings were so arranged that some of the lateral branches were free between the rings. In nearly every instance

<sup>18</sup>Some hydroids react more quickly and more readily to contact than do other hydroids, Campanularia and Pennaria more than Eudendrium. See Loeb '91.

the lateral branches within the tube were in contact with the glass. The results were very decisive.

TABLE 18

Polyps regenerated on	3 days	6 days	No. branches removed
Cut ends, within the tube .....	0%	0	88
Cut ends, outside of tube .....	56%	116%	34

In no case did regeneration occur on cut ends within the glass tubing. After six days these stems were removed from their tubes, and regeneration then proceeded normally at nearly all the cut ends.

*Experiment 19.* Other experiments under somewhat different conditions illustrate further the inhibition due to contact. Each stem was rolled in a thick sheet of cotton, which was then immersed in water. No hydranths regenerated. Cotton about half as thick was used and several hydranths appeared in three days, but by the sixth day they were gone and did not reappear thereafter. By using cotton one-half as thin again, a still larger number of polyps were produced, which disappeared less rapidly than in the preceding case. The results were similar when stems were placed between two flat layers of cotton, so arranged that a stream of sea-water continually flowed through the cotton. When removed from the cotton, the stems regenerated readily—provided they had not been kept too long in it.

*Experiment 20.* On one side of a dish of sea-water stems were loosely placed, on the other side a larger number of stems were crowded together into a groove 4 mm. wide by 5 mm. deep and somewhat longer than the length of the stems. Table 20 gives the detailed results.

TABLE 20

		Indicates the total number of polyps observed on the following days									
		3d	4th	5th	6th	7th	8th	9th	10th	11th	No. of stems
1	Crowded stems . . . . .	2	2	1	2	0	0	2	1	2	10
2	Not crowded stems . . . .	10	23	10	2	3	6	6	9	16	6
3	Crowded stems . . . . .	20	15	7	4	4	6	10	12	39	* 12
4	Not crowded stems . . . .	40	35	20	27	36	22	20	20	19	

\*Stems had been scattered.

The crowded stems regenerated only at the free unentwined branches on the top of the pile, and regeneration was less than in

the scattered stems. When the crowded stems were removed from the groove and separated from one another, there was a markedly increased regeneration.

*Experiment 21.* Dr. Louis Murbach kindly permitted me to use an ingenious apparatus devised by him, by means of which a stream of air bubbles was introduced at the bottom of a vessel so that the contained water was in constant agitation. Stems placed in the vessels were whirled around making about 35 revolutions per minute in one vessel and about 28 in the other. Very little regeneration occurred (about 8 per cent), somewhat more in the slower stream, slightly less in the faster one. Two days after the appearance of polyps they were gone, and no more regeneration occurred. If the current of bubbles was stopped for 12 to 24 hours, some regeneration would take place. After ten days the stream was stopped altogether, and there resulted a constantly increasing number of hydranths. Whirling stems through water at a comparatively rapid rate affects regeneration in practically the same manner as contact, already discussed.

*Contact is unfavorable to and more or less suppresses the development of polyps. It matters little whether branches touch each other, or collide with a solid object, or whether contact is due to growth within a confined space.* In the latter case there is an increasing pressure proportional to the amount of growth. Contact may be reinforced by pressure resulting from the weight of a superimposed layer of wet cotton; or contact may be the impact resulting from the whirling of stems through water. *Whatever the nature of the contact or pressure or weight or impact, regeneration of polyps is inhibited in proportion to the degree of pressure, weight, impact, etc.*

#### EFFECTS OF LACK OF OXYGEN<sup>19</sup>

*Experiment 22.* Sea-water was boiled to remove the oxygen more or less completely from it and the amount of water evaporated was replaced by an equal quantity of boiled tap water. An equal number of stems was placed in flasks filled to the brim with this deoxygenated sea-water. The smallest possible space

<sup>19</sup>Loeb '91 and '95, *Plüger's Archiv.*, vol. 62.

was left between the water and the cork, then the flask was hermetically sealed. The following tables indicate in per cent the volume of water after boiling. These figures will serve in a general way to indicate the amount of oxygen removed.

TABLE 20a

	No. of hydranths observed on the following days									No. of stems
	2d	3d	4th	5th	6th	7th	8th	9th	10th	100
1 Norm sea-water in open dish.....	40	18	8	7	8	11	13	8	6	
2 Norm sea-water in sealed vessel .....	14	13	12	3	5	6	5	0	0	
3 to 10; from 97% to 77%	0	0	0	0	0	0	0	0	0	

*There was absolutely no sign of regeneration in the eight sealed flasks, Nos. 3 to 10 inclusive, from which the oxygen had been removed from the sea-water and its reabsorption prevented.* Flask No. 2 which was also hermetically sealed, contained normal sea-water, and regenerated fewer polyps than the open dish No. 1. Regeneration in the former ceased altogether after eight days, while in the latter polyps continued to be produced. Regeneration in the hermetically sealed flask No. 2 was inhibited, either because the supply of oxygen in the water was entirely appropriated by the previously developed polyps or because of the carbon dioxide produced by these polyps, or by both of these causes acting together.

Additional evidence of a very interesting nature was obtained by filling wide mouthed jars about two-thirds full of oxygen-free sea-water as above. There was, however, a layer of air over one inch deep between the water and the cover, which was sealed airtight.

TABLE 20b

No.	No. of min- utes boiled	Evap- orated to	No. of hydranths observed on the following days											
			3d	4th	5th	6th	7th	9th	10th	11th	12th	13th	14th	
1	control	control	8	2	3	7	18	7	7	2	3	3	4	
2	4	98%	2	0	9	11	7	2	3	2	2	1	2	
3	8	82½	4	4	9	7	4	2	4	3	3	5	6	
4	10	81	1	0	0	3	8	2	3	0	0	0	0	
5	12½	75	0	0	2	0	0	1	1	0	0	0	0	
6	15	73*	1	0	1	6	5	7	8	2	2	2	0	
7	17½	70	0	0	2	2	2	3	2	2	1	0	0	

\* Probably an error.

Polyps were produced in all the jars. The greatest number appeared in the control dish No. 1. The previous experiment showed that no polyps are produced in water from which the oxygen had been removed. But in this experiment the water reabsorbed enough oxygen from the overlying layer of air to supply the needs of the developing polyps. Where large quantities of oxygen had been removed from sea-water as in Nos. 4, 5, 6 and 7 a much longer time was required to absorb enough oxygen to permit regeneration to begin; and the available supply of oxygen was more quickly exhausted in these than in Nos. 2 and 3, therefore, regeneration ceased earlier in the former series than in the latter. It follows that *where much oxygen has been removed from sea-water, polyps appear later and disappear earlier than on stems kept in water containing more oxygen.*

#### EFFECTS OF DIRECT SUNLIGHT<sup>20</sup>

*Experiment 23.* This experiment unfortunately was not carried to completion. The apparatus was so placed that the sun shone directly upon the stems for about eight hours daily. The heat of the sun was guarded against by reducing radiation from the table and fixtures to a minimum and by surrounding the dishes by large volumes of water to which ice was sometimes added. Temperature records of each of the dishes were made at least three times daily. The stems were grouped as follows:

- 1 Dish was not guarded against the heat of the sun.
- 2 Dish was surrounded by 3000 cc. of water.
- 3 Dish was surrounded by 9000 cc. of water.
- 1, 2 and 3 were exposed to the direct rays of the sun.
- 4 Not exposed to the light, but kept in the shade of the room.
- 5 Not exposed to the light, but kept in a dark chamber.

The temperature in 1 was, of course, several degrees higher than in any of the other dishes, especially about midday, when the temperature was often as high as 28° C. In 2, the temperature was lower, while in 3, 4 and 5, which were practically the same, the temperature was lowest.

<sup>20</sup>Loeb '92, '96; Driesch Zoöl. Jahrb. '90; Goldfarb '06.



The greatest regeneration occurred in 1, then came 2 and 3, which regenerated about the same number and less than 1; there was a decided drop in 4 and 5 which regenerated least. There was a notable exception in one of the dishes of series 5 in which a surprisingly large number of polyps were produced. *Direct sunlight stimulates stems to increased regeneration of polyps*, even though the temperature of the water in which they are contained rises as high as 28°C. There is, furthermore, an undoubted positively phototropic bending of some of the new polyp-bearing branches.

#### EFFECTS OF TEMPERATURE<sup>21</sup>

*Experiment 24.* In the previous experiments bacterial increase was guarded against, particularly in the higher temperatures, and hydranths prospered in a temperature as high as 28° C. Without this precaution such high temperatures would be fatal to the stems. At what higher temperature polyps would be regenerated was not determined. Up to a certain point the greater the warmth the greater the regeneration.

When stems were placed in a refrigerator in which the temperature varied from 10° to 16° C. there was no mistaking the inhibitory effects of the cold. A large number of stems never regenerated at all, and the total number produced was exceedingly small.

#### EFFECTS OF REPEATED REMOVAL OF POLYPS FROM THE SAME LATERAL BRANCHES<sup>22</sup>

*Experiment 25.* As soon as polyps were distinctly differentiated at the cut ends, they were again amputated. This daily removal of polyps was carried on for a period of 31 days, and daily records made of the number and position of the polyps removed. During this time there were regenerated:

- 1 At 15 cut ends, including oral and basal, 59 different polyps;
  - 2 At 11 cut ends, including oral and basal, 28 different polyps;
  - 3 At 15 cut ends, including oral and basal, 64 different polyps,
- making a total of 41 cut ends regenerating 151 new polyps or 368 per cent in 31 days.

<sup>21</sup>Peebles '98, on The Effects of Temperature on Reg. of Hydra, Zool. Bull.

<sup>22</sup>Hargitt, G. T., '03, Reg. in Hydromedusæ, Archiv f. Ent. '03.

Many cut ends regenerated but once during this entire period, others as many as ten times. The greater or less regeneration was not confined to any definite region of the stems. The oral ends rarely produced any polyps for reasons already given. The stems continued to regenerate polyps normal in every regard, until the last days of the experiment when they decreased appreciably in size. Though observations ceased at the end of 31 days, regeneration of polyps would most likely have continued further.

#### EFFECTS OF OTHER INJURIES TO STEMS

If a stem or branch is cut at any level and the cut end is exposed to sea-water, a hydranth is normally produced. The question arose whether any severe injury except cutting a stem completely across would result in the formation of a polyp at the point of injury, and whether the regeneration of such adventitious polyps would effect regeneration at the neighboring cut ends.

*Experiment 26.* All the internodes of several stems were either bored through with a needle, or severely lacerated or slit with a fine scissors. No regeneration at the injured internodes occurred except in two instances. The wound appeared to close up immediately, only a crack in the perisarc marked the place of injury. In the two instances, above noted, the hydranths grew out at right angles to the stem, in marked contrast to the hydranths on the rest of the stem, all of which pointed orally. The large number of injuries on each stem did not reduce the number of lateral, oral or basal hydranths regenerated. Nor did the bending of large stems permanently into an acute angle, effect regeneration at any of the cut ends. When, however, one or two internodes on each stem were slit and the stems then bent so that the wounds were kept exposed to the sea-water, a large number of hydranths appeared from the bent ends. It made no difference whether the bend formed an acute or right angle. Nor was regeneration at any of the other cut ends, including oral and basal, effected by the formation of these adventitious hydranths.

The cœnosarc from each of the two injured ends of a slit would grow out directly in line with the axis of the stem, and then fuse into a single branch which would regenerate a hydranth at the

distal end. Sometimes the cœnosarc from the two ends would fuse close to the wound, or each wounded end may independently regenerate a polyp, or less frequently the one or the other end only, develops a polyp. Whether the injury was at the distal, middle or basal part of the stem did not influence the regeneration.

TABLE 21

No. of stems experimented upon	Nature of the Operation	No. polyps reg. at injured ends in 6 days
6	Stems punctured or slit at every internode	1
8	Stems lacerated at every internode	2
6	Stems bent permanently	0
4	Stems bent and punctured at the bend	0
12	Stems slit and bent at point of injury	11

*If a sufficiently large area of cœnosarc is cut, irrespective of the level of the stem, and the wounds are prevented from closing immediately, a relatively large number of hydranths are regenerated. These adventitious polyps appear at the same time as the polyps on the lateral branches, and seemed in no way to effect the regeneration of the latter.*

*Experiment 27.* After stems had been experimented upon for a long time and could no longer be made to regenerate, they were cut into small pieces. Sometimes the lateral branches were also cut close to the stem. The pieces cut from the distal regions never regenerated, for the very obvious reason that cœnosarc is never present in the distal parts of stems. But the middle and basal pieces regenerated an incredibly large number of polyps at their cut oral and basal ends. Some of the lateral branches which had been cut close to the main stem also regenerated polyps. Here again injury to the cœnosarc accompanied by exposure to sea-water rejuvenated the pieces in so far as rapid and extensive regeneration of polyps is concerned.

#### EFFECTS OF DILUTED AND CONCENTRATED SEA-WATER<sup>23</sup>

*Experiment 28.* Sea-water was diluted by the addition of tap-water so as to make a graded series, with differences of 5 and sometimes 10 per cent, from normal sea-water to 50 per cent dilution.

<sup>23</sup>These experiments are based on Loeb's '91. See also Snyder '05, *Archiv. f. Ent.* Large numbers of stems and branches were used.

In the experiments of 1905 the total number of polyps was daily recorded. These records agree with those of Loeb, that the number regenerated increases with the increased dilution of sea-water. The maximum regeneration is reached in sea-water diluted 15 to 20 per cent; beyond this point, that is, in solutions more diluted, regeneration rapidly decreases; in 40 per cent, few polyps are produced, in 50 per cent, none.

In 1906 the experiment was repeated, but the records indicate the number of *different* hydranths daily regenerated. The results are practically in accord with the data obtained by the other method in 1905. The rate of development in both series was the same in normal sea-water and in solutions diluted as much as 15 per cent but beyond this point the greater the dilution the greater the retardation.

TABLE 22

Solution	No. of different polyps regenerated in		No. of branches used
	3 days	6 days	
Normal	46%	82%	234
5%	56%	79%	
15%	62%	84%	
25%	28%	73%	
35%	0%	34%	

Stems of *Pennaria tiarella* behave in quite the same manner as *Eudendrium* in dilute solutions of sea-water. The results in both hydroids are practically the same.

*Experiment 29.* An effort was made to acclimatize the stems of *Eudendrium* and *Pennaria* to greatly diluted sea-water, and thereby to have them regenerate in solutions diluted 50 per cent or more. Sea-water was daily diluted  $2\frac{1}{2}$  per cent more than the preceding day. Polyps appeared in all dilutions until 45 per cent was reached, beyond which regeneration ceased on *Eudendrium* stems, while *Pennaria* ceased at 50 per cent. In the very diluted solutions polyps were distinctly smaller than the normal polyps. The experiment seemed to show that hydranths of *Eudendrium* and *Pennaria* could not be made to regenerate in solutions diluted more than 45 and 50 per cent, respectively, during the 25 days of the experiment.

*Experiment 30.* The stems from the preceding experiments were removed from their solutions to normal sea-water. Four to six days after the transfer, hydranths appeared, first, on stems from the least diluted sea-water, later on stems taken from 20 to 30 per cent dilutions while no regeneration occurred on stems kept in water diluted 40 per cent or more. The number regenerated increased daily but not to the same degree, so that by the eleventh day after the transfer the stems taken from the greatly diluted water regenerated as much as those taken from the normal or slightly diluted sea-water.

TABLE 23

Solution prior to transfer	Stems in dilute solutions for 11 days were transferred to normal sea-water			
	Per cent regenerated in			
	3 days	6 days	9 days	11 days
Norm	9*	20	32	40
5%	0	9	21	28
10%	0	22	36	47
15%	16*	22	32	38
20%	0	3	15	30
25%	0	10	14	27
30%	0	5	22	33
35%	0	6	26	40
40%	0	0	0	0

\* These polyps were present at time of transfer.

The effects of concentrated sea-water will be taken up more fully in the following experiments.

*Experiment 31.* A graded series was made by boiling and thereby concentrating sea-water. For example, 200 cc. of sea-water at 18° C. when boiled to a volume which at the original temperature was 180 cc. constituted a 90 per cent concentrated solution. The water was filtered and aerated by thorough shaking. Every few days the water was replaced by water freshly concentrated to about the same per cent.

TABLE 24a

Concentration	Polyps regenerated on the following days:						No. of branches 650
	2d	3d	4th	5th	6th	7th	
Norm	11%	50%	55%	52%	32%	5%	
92% to 95%	0	5	15	15	15	$\frac{1}{2}$	
85% to 89%	0	2	6	12	12	10	
77%	0	0	0	1	0	0	
65%	0	0	0	0	0	0	
62%	0	0	0	0	0	0	

TABLE 24b

Concentration	Polyps reg. in		No. of branches
	3 days	6 days	
Norm	51%	91%	719
90%	14	51	
87%	7	32	
85%	5	37	
80% } 76% } 70% }	0	0	
etc			

The more concentrated the sea-water the fewer the polyyps produced. Slight increase of concentration results in large decrease in the number regenerated. The maximum concentration beyond which no regeneration occurs is about 77 per cent. Retardation observed in diluted sea-water likewise takes place in concentrated solutions. It is almost needless to add that the greater the concentration the slower as well as the fewer the polyyps produced.

*Experiment 32.* The maximum concentration at which regeneration of Eudendrium stems will take place was more definitely ascertained by transferring them daily to solutions more concentrated by about  $2\frac{1}{2}$  per cent. Regeneration occurred in all concentrations from norm to  $57\frac{1}{2}$  per cent. Even in 50 per cent solutions, shoots were observed. Thus by slowly increasing the concentration of sea-water, stems were made to regenerate polyps in a considerably greater concentration than had otherwise taken place, when stems were placed at once into the  $57\frac{1}{2}$  per cent concentrated solution.

*Experiment 33.* Stems that had been kept in concentrated solutions were after 18 days transferred to normal sea-water.

TABLE 25

Stems kept in concentrated solution 18 days were transferred to normal sea-water. On the following days there regenerated:

[illegible]

The greater the difference in salinity between the solutions in which stems had been kept and normal sea-water, the greater the regeneration after the transfer. But this is exactly what might have been expected from Experiment 28. Seventy-nine per cent concentration or thereabouts, marks the toxic point beyond which stems do not regenerate when transferred to normal sea-water.

The percentage of salts present in sea-water determines whether regeneration shall or shall not take place. An excess, or on the contrary, too little salts present in the solution prevents regeneration. Whether the effects produced are the result of differences in osmotic pressure or of the specific action of the salts or of both of these factors was not determined. *Regeneration took place on the one hand in solutions diluted to 45 per cent and on the other concentrated to 58 per cent. From these two extremes the number regenerated increases to a maximum not in normal sea-water but in 15 to 20 per cent diluted sea-water.*

#### CONCLUSIONS AND SUMMARY

It is extremely difficult, even approximately, to distinguish the external from the internal factors in regeneration. Both kinds of factors play important rôles in the life history of Eudendrium and other hydroids. For convenience and for purposes of study each of the factors in these two series were separately considered, though it should be remembered at all times that this is an artificial though convenient arrangement, and that these factors never act singly and independently of the rest. These influences bring about various reactions, only some of which may be said to be adaptive. The following five factors may be said to result in adaptive changes in Eudendrium.<sup>24</sup>

1 *Gravity* determines the position, at which regeneration shall more frequently take place, and the direction of growth. It does not determine the kind of regeneration, for with rare exceptions only polyps are produced when regeneration occurs at all. On erect stems oral and lateral cut ends regenerate profusely. On inverted stems, regeneration is greatly stimulated,

<sup>24</sup>Loeb laid emphasis on but two factors, namely, light and gravity.

at the basal end only, while polyp formation at the lateral and oral ends is largely inhibited. New stems and branches show a strongly negative geotropism, and grow upward irrespective of the position of the piece.

2 *Sunlight.* Stems or branches exposed to the direct rays of the sun regenerated a greater number of polyps than those kept in the shade of the room, the temperature in both cases being approximately the same. How much the increase was directly due to the effect of the actinic rays *per se*, or indirectly to the destruction of bacteria, or to the slightly increased temperature, or to all of these factors was not ascertained. Many of the stems and branches bend toward the sun, *i. e.*, they are positively heliotropic.

3 *Temperature.* Other conditions being favorable and equal, regeneration increases with increased temperature to the optimum, and decreases with the lowering of the temperature. At 10° C. regeneration is largely inhibited, while regeneration increases up to and including 28° C. temperature. One of the more important conditions, just mentioned, is exposure to sunlight for stems placed in water at a moderately high temperature and not exposed to direct sunlight produces far fewer polyps.

4 Any *severe injury* at any level of the colony, may cause polyps to regenerate, if the wound be exposed to sea-water. The direction of growth of the pedicels and the rate of development of the polyps are subject to the external conditions mentioned and to the internal conditions to follow.

5 *Contact*, pressure, impact, etc., are inhibiting influences which tend to prevent complete development at those ends that come in contact or are pressed upon by a solid body. Shoots are often produced, but further differentiation is stopped. Contact determines, in some degree, particularly on very young branches, the direction of growth, which is away from any solid body, therefore, is negatively stereotropic. The amount of inhibition is proportionate to the degree of contact, pressure, impact, etc.

6 Large variations in the concentration of sea-water probably never occurs in nature and the reactions of Eudendrium to differently concentrated solutions can hardly be called adaptive. The maximum number of polyps regenerated does not occur in normal



sea-water but in solutions diluted with about 20 per cent of tap-water. The amount of salts present in this solution is most favorable to regeneration. As the quantity of salts is increased by concentration or decreased by further dilution, the number regenerated decreases until the minimum is reached on the one hand at 45 per cent dilution and on the other at 58 per cent concentrated sea-water. Stems transferred from concentrated to norm sea-water which is equivalent to placing them in dilute solution, regenerate according to the principle laid down, viz: the more dilute the solution, to a certain point, the more hydranths produced. On the contrary, stems transferred from dilute to norm sea-water, which is practically placing them into more concentrated sea-water, do not regenerate less than stems continuously kept in normal sea-water. Regeneration is not inhibited until the solution contains more salts than that normally present in sea-water, while the stimulating effects of diluted sea-water occurs, when either concentrated, normal or dilute solutions are diluted to what is equivalent to a 20 per cent dilute sea-water.

Before summarizing the internal factors, the behavior of the cœnosarc under different conditions might perhaps more profitably be taken up.

The cœnosarc is circular in cross section, with no partition as in the case of Tubularia. Granules within the cœnosarc stream alternately toward the apical and basal ends, either in the same direction throughout the colony or independently, in each branch, or even in different directions in the main stem. The cœnosarc itself can move *en masse* within the perisarc. *With the basal two-thirds of stems excepted, the cœnosarc invariably moves toward the basal end of the piece, i. e., in all branches, in the apical pieces of stems, and in small pieces from any region of the colony. It may even move entirely out of the piece, through the basal end. Now, as regeneration occurs only where the cœnosarc is present, it follows that whether regeneration shall or shall not take place at a cut end is determined by the migration of the cœnosarc. This movement basally crowds or concentrates the cœnosarc at the basal end of the piece and if conditions are favorable at the end polyps readily appear there. The migration of the cœnosarc may be furthered in various ways, namely:*

1 Inverting pieces is almost certain to stimulate regeneration at basal ends; or, better still, embed inverted pieces in sand and a remarkable number of basal polyps appear on the free parts.

2 Tying a ligature at the distal end of a stem or, still better, ligature the lateral branches, then ligate the middle of the stem, and a greatly increased number of heteromorphic polyps result.

3 By cutting small pieces from any part of the colony a far greater number of polyps is produced at the basal than at the apical ends.

*Thus though the cœnosarc is influenced by such external factors as gravity, ligatures, lack of oxygen, cold, and by internal factors, such as age, size of the piece, etc., the cœnosarc normally behaves in certain definite ways which, without the aid of hypothetical "specific stuffs" not only accounts for the absence of polyps at certain cut ends but accounts for their regeneration at other ends. Under a given set of conditions we can foretell with a fair degree of accuracy the number and region at which regeneration will take place.*

Furthermore, it is not necessary to have recourse to the stimulating effects of necrotic tissues thrown into the circulation to account for the regeneration of polyps. In *Tubularia* it has been maintained that the breaking down of the partition near the cut end throws into the circulation material which stimulates regeneration at that end. In a hydroid resembling *Eudendrium*, namely, *Pennaria*, Gast and Godlewski believed that the disintegration of polyps supplied the circulation with material which stimulates regeneration. In *Eudendrium* there is neither a partition as in *Tubularia*, nor were the polyps permitted to disintegrate, for they were cut off at the beginning of the experiment. Yet hydranths were formed within 48 hours often at every cut end. Even when hydranths were daily removed as soon as formed, other polyps were regenerated.

The following internal factors affect regeneration:

1 *Age* determines not the kind but the rate and number regenerated. The younger the region the more numerous and the quicker do polyps appear. This statement is subject to special conditions already enumerated, such as ligatures, inversion of stems, migration of the cœnosarc, etc.

2 *The influence of the presence or absence of lateral branches and their pedicels.* Pieces with the lateral branches cut off close to the main stem regenerate many more polyps than similar stems from which only the polyps have been removed, for the reason that the cœnosarc tends to withdraw from the pedicels whereas it does not do so from the lateral ends cut close to the stem.

3 This suggests another closely related factor, viz: *the influence of size (i. e., the amount of cœnosarc) on the number and position of the regenerated polyps.* Pieces less than 1 mm. long may regenerate stems but never complete polyps. Pieces 1 to 1½ mm. long regenerate but one polyp, more frequently however at the basal end. Sometimes one is produced at each end. On larger pieces two or three polyps may appear on an outgrowth at the basal end. Still larger pieces may bear as many as nine basal polyps at one time, while it is rare for more than one apical polyp to be produced.

4 *The influence of the old tissue on the kind of regeneration.* Polyps are replaced only by polyps; stems if injured give rise to polyps. Under certain unfavorable conditions proliferation of cells may take place but no differentiation into polyps occurs, and "stolons," or modified stems, result. If these are cut and removed or grow into a favorable environment polyps, not "stolons," are regenerated.

The *rate of regeneration* varies with the size and age of the piece. Large stems produce polyps quickest at the distal, slowest at the basal region. Medium size pieces regenerate at all the cut ends at the same time. Polyp formation is greatly retarded on small pieces even if the pieces are taken from the distal region of large stems. The two cut ends at any level of a stem regenerate at the same time. The presence of polyps does not prevent, but may retard, regeneration at the basal end. Unfavorable conditions, such as lack of oxygen, low temperature, greatly diluted and even slightly concentrated sea-water, gravity (on lateral ends of reversed stems) all retard development. Nothing availed to affect regeneration in less than two days.

## STUDIES ON REGULATION

### XI FUNCTIONAL REGULATION IN THE INTESTINE OF CESTOPLANA

BY

C. M. CHILD

WITH TWENTY TEXT FIGURES

This Neapolitan form which has served for other experiments (Child '05a, '05b, '05c) is very favorable for the study of the intestinal changes which occur during form-regulation. The intestine in normal animals is almost black in color and since other portions of the body are unpigmented is very distinctly visible in the living animal. Moreover, the regulatory changes are extreme and in some cases relatively rapid; and finally animals and pieces live for months in clear water without food so that it is possible to follow the intestinal changes during a long period.

#### I THE TURBELLARIAN INTESTINE, ITS FUNCTIONS, AND FUNCTIONAL FACTORS INVOLVED IN ITS DEVELOPMENT AND REGULATION

This part of the paper aims to establish a general basis for interpretation of the experiments and observations to be described later. It precedes rather than follows the descriptive part because it is important, as well as economical of time and space, to be able to point out the bearing of the various experimental data, under each head instead of postponing interpretation to a general section where the chief points of the description must be reviewed.

The basis of interpretation suggested here is, however, in part the result of these and other similar experiments, not a preconceived hypothesis with which the facts are to be brought into accord. As will appear also, it is in line with previous suggestions which I have made concerning the dynamic or functional character of form-regulation (Child '05a, '06a, '06b).

*I The Turbellarian Intestine and its Functions*

The names by which the various organs of the lower invertebrates are designated do not necessarily serve to indicate with any degree of exactness their functions. We commonly speak of the alimentary apparatus of such forms as the turbellaria as an intestine, a digestive system, etc., but strictly speaking the functions of this apparatus are not identical in all respects with those of the intestine of the vertebrates for example. It is of course a digestive system, but it is more than that.

In the first place, the turbellarian intestine undoubtedly serves as a place of storage for undigested nutritive material. Any one who has observed turbellaria feeding can scarcely fail to recognize that this is an important function of the intestine, at least in certain species. Food is often taken until not only the intestine but the whole body is greatly distended. In fact I have often observed the bursting of various species in consequence of rapid intake of food. The opening in such cases is usually small and after outflow of the excess of material soon closes. Under such conditions the intestinal walls must of course undergo great mechanical extension.

Secondly, digestion is, at least in part, intracellular and the intestinal cells undoubtedly accumulate reserve material when food is abundant; in other words, when digestion proceeds more rapidly than material is removed.

But besides the functions of digestion and accumulation of reserves the intestine in these forms is the chief means of distribution of the nutritive material to various parts of the body, *i. e.*, it is in greater or less degree a circulatory system, a fact which has been recognized by those authors who have termed it the gastro-vascular system. As a gastro-vascular system it contains fluid laden with nutritive substances. This fluid moves to and fro, enters and leaves the various branches and regions according to the muscular contractions of the body-wall. Thus the intestinal wall is subjected to the varying fluid pressures which, however, are more or less typical for each particular region since the muscular contractions are in general typical. A wide range of conditions

exists, of course, for each region, but the conditions in the terminal regions, for example, must be in general typically different from those in the middle region.

To sum up: the turbellarian intestine as an organ of digestion and a store-house of reserve material is undoubtedly the seat of typical chemical reaction-complexes. As a reservoir for the temporary storage of undigested food and as a vascular system containing moving fluid it is undoubtedly subjected to a typical complex of mechanical conditions. These two groups comprise, I believe, the most important functional conditions for the turbellarian intestine.

The intestine of higher forms, or at least some part of it, serves as a place of temporary storage for undigested food and often, as in certain birds and mammals, undergoes a high degree of specialization in connection with this function. But in higher forms where a specialized circulatory system is present, the intestine does not function to any great extent as a system for the distribution of nutritive material and is not subjected to the mechanical conditions which must exist in such a system, although of course mechanical functional conditions are more or less important factors in the functional complex in all cases. There can be no doubt, however, that mechanical conditions constitute a much larger element in the functional complex characteristic of the turbellarian intestine than they do in higher forms. If functional factors play any part in development and regulation, we may expect to find the determining factors in the two cases different to a greater or less extent.

## *2 Functional Factors in Intestinal Development and Regulation*

It is a well-established fact that the mechanical conditions connected with the movements and pressure of fluid within the vessels are factors of great importance in determining diameter, distribution, angle of branching and character of the wall of the blood-vessels. Since this is the case it is natural to expect that similar conditions will play a rôle of greater or less importance in development and regulation of the turbellarian intestine.

Judging from the form of the turbellarian intestine in relation to the form and structure of other parts it is difficult not to believe that functional and particularly mechanical conditions are important factors in its development. In the rhabdocœls where no strands of parenchyma or dorso-ventral muscles oppose it, it forms simply a sac, filling the pseudocœl in part or wholly according to conditions. In the polyclads, on the other hand, the intestine might be compared roughly to an elastic sac placed in a space in the axis of the body and then gradually distended so that parts of it are forced into the parenchymal spaces toward the periphery of the body. If the fluid contents of the intestine move and exert pressure in typical directions it seems to me that the effect of these movements must necessarily appear in the direction and size of the intestinal branches. All the facts seem to indicate that the general direction and arrangement of the intestinal branches in the various parts of the body is determined, at least in large part, by the mechanical conditions resulting from movements and pressures of the fluid contents. By altering these conditions the arrangement of the intestinal branches can be altered, as I showed for *Leptoplana* (Child '04a). In the triclads conditions are similar but the intestine develops in different form because of the position and form of the pharynx. The almost infinite variations in type of the "normal" turbellarian intestine in a given species simply show, in my opinion, how largely its form as regards details is a matter of chance, determined often by the presence, absence, or position of spaces, or dorso-ventral muscular fibers in the parenchyma, by slight individual differences in movement or constitution of other parts, etc.

If the functional conditions connected with the movements and pressures of fluid contents are essential factors in determining the form of the intestine, we may expect to find changes of form occurring when these factors change and the facts justify our expectations. Starvation of a planarian results in degeneration and total disappearance of the most distal portions of the intestine in succession: feeding results in the redevelopment of branches, but not necessarily in the same pattern, and increased distension of a normal animal results in the formation of new intestinal branches.

But it is in connection with experiments on form-regulation that the extreme plasticity of the turbellarian intestine becomes evident. The changes in form and arrangement of the intestinal branches in the experiments of Lillie ('01) and Bardeen ('01, '02, '03) are sufficient to illustrate this point, although they do not demonstrate its correlation with the functional conditions resulting from the movements and pressures of fluid contents.

In most triclads and polyclads intestinal regeneration is usually much less complete than the regeneration of other parts when the animals are not fed. Moreover, and this seems to me to be a crucial point, it is much less complete in pieces without the cephalic ganglia than in pieces containing the ganglia (Child '04a). It can scarcely be supposed that there is any essential difference in nutritive conditions between pieces with and those without the ganglia. If anything, more nutritive material should be available for growth in the piece without ganglia since it is much less active than the other. I do not believe, however, that such differences in intestinal regulation can be due primarily to the differences in nutritive conditions. The only reasonable basis for interpretation seems to me to lie in the differences in activity. In the piece without ganglia the movements of the intestinal contents are less frequent and less energetic, and consequently the stimulus to intestinal growth in the new tissue is less than in the piece containing the ganglia. Observation of two such pieces and of the movements of intestinal contents in their bodies shows very clearly that the intestinal pressures and tensions are much greater in the piece containing the ganglia than in that without them.

All the data thus far available seem to me to indicate that the form and arrangement of parts of the turbellarian intestine is determined very largely by mechanical factors due to the presence and movements within it of fluid contents. This statement is not to be interpreted, however, as signifying that nutritive factors play no part in determining intestinal form. The form must be altered to a certain extent by the presence or absence of reserve material in the cells, by the general metabolic conditions, the relation between intake and output, etc. But I find it difficult to understand how such factors as these can possibly determine the general



outline of the intestine, and the direction and arrangement of its branches. Lack of nutrition may of course determine the degeneration of a branch or of branches, but how can the presence of nutrition determine the position and direction of new branches? On the other hand, the conditions above mentioned do account readily for position, outline, and arrangement of parts and experimental data indicate that they are the factors chiefly involved.

The development of intestinal branches is simply another illustration of the fact which I have mentioned elsewhere at various times, viz: that the stimulus to growth is not identical with the presence of nutritive material, but that, on the other hand, nutritive material goes where the demand is greatest even at the expense of reduction and disappearance of other parts where the demand is less. This relation between growth and nutrition seems also to show why such extensive intestinal reduction occurs in many turbellaria during starvation: the demand for nutritive material is greater in other parts than in the intestine, consequently material passes from it to them. In short, I believe the whole problem of the "self-regulation of metabolism" during starvation and indeed at other times is essentially a problem of relative functional activity in the broadest sense.

In *Cestoplana* the axial intestine extends directly through the median region of the body from end to end. The lateral branches are at right angles to the axial intestine in the pharyngeal region, but toward the anterior end gradually change their direction, and are directed more and more anteriorly: posterior to the pharynx exactly the reverse is the case (Fig. 1).

The movements of intestinal contents in this species are briefly as follows: general contraction of the body forces the intestinal contents from both ends toward the pharyngeal region, and the axial intestine and the lateral branches of the middle region of the body become distended. General extension of the body forces the intestinal contents out of the middle region to a large extent and distributes them along the lateral branches even to the extreme terminal regions, if the contraction is strong.

Under these conditions the intestinal contents move anteriorly in the prepharyngeal and posteriorly in the postpharyngeal region.

Local contractions and extensions of course cause local changes in the distribution of intestinal contents, but these follow the same rules as the more general movements.

Evidently then, the intestinal branches in the middle region are filled and distended by the intestinal contents which accumulate in the middle region during contraction and the branches in the terminal regions by the contents during their flow away from the middle region. In the regions between the middle and end all intermediate conditions exist. Those branches which are filled and distended chiefly by the fluid accumulating in the pharyngeal region arise at right angles to the axial intestine since their formation is correlated essentially to lateral pressure of the intestinal contents, escape in other directions being impossible. But toward the ends of the body the intestinal branches are filled and distended by fluid which is moving anteriorly or posteriorly. If mechanical conditions are factors in determining the form of the intestine, the intestinal branches in these regions may be expected in accordance with the laws of hydrodynamics to be directed more or less obliquely in the direction in which the fluid is moving. The intestine in *Cestoplana* seems to me to possess exactly the form which might be expected if movements and pressures of fluid contents are the chief factors in producing it. The fact that a gradual change in direction of the branches between the middle and the ends of the body exists is due simply to the gradual change in conditions. In the regions between the middle and terminal regions the branches are filled and distended in part by the fluid moving away from the pharynx, and in part by standing contents escaping laterally from pressure in other directions. The nearer the pharyngeal region, the more exclusive the latter condition of filling and distension, the farther away the more exclusive the former. Hence we may expect to find with increasing distance from the pharynx a gradual change in the direction of the branches from a position at right angles to the axis to one oblique toward the direction of movement of the contents.

Similar conditions in general, with of course various specific differences, exist in other polyclads and triclads, and the form of the intestine as a whole and of each of the long branches in many

of the broader polyclads corresponds very closely to what may be expected if hydrodynamic factors play an important part in their formation.

On the following pages the various regulatory changes in the intestine of *Cestoplana* under various conditions are described and their bearing on the above dynamic hypothesis of intestinal development is discussed.

Perhaps it should be added in order to forestall objections that hydrodynamic factors are not considered as the only factors involved in determining intestinal outline and arrangement of parts in the turbellaria. It seems very probable that other factors must also play some part, though the facts seem to me to indicate that hydrodynamic factors are certainly of great importance.

## II THE NORMAL INTESTINE AND THE TYPICAL COURSE OF INTESTINAL DEGENERATION IN THE ABSENCE OF FOOD

### *I Descriptive*

The appearance of the intestine in newly captured animals differs to some extent, apparently according to the previously existing conditions. In Fig. 1 the terminal and middle regions of the intestine in a normal newly captured specimen are shown, somewhat diagrammatically. The intestine in this case is only moderately distended by its contents: in many cases it is so distended that no spaces between the branches are visible and it appears as in Fig. 2.

In uninjured animals kept without food a gradual reduction or degeneration of the intestinal branches occurs, though much more slowly than under certain experimental conditions. Intestinal reduction proceeds from the peripheral or terminal region of the intestine toward the middle.

The first parts to disappear are the tips of the branches at the anterior and posterior end and as reduction of these branches continues branches nearer the middle region are affected until a condition resembling that shown in Fig. 6 is attained. In this case which represents a normal animal after about four and a half months without food, only short stumps of the lateral branches

remain in the terminal regions of the body. With approach toward the middle the length of the branches increases until in the pharyngeal region they still retain their full length, though they are less distended than originally. At the beginning of the experiment the intestine of the specimen figured presented the condition indicated in Fig. 2.

Undoubtedly intestinal reduction could be carried further in normal animals, but departure from Naples made it impossible to keep the specimens under observation longer.

Intestinal reduction in this species consists in an atrophy and disintegration of the more distal portions of the intestine, not merely in a reduction in size or contraction. Various stages can be more or less clearly distinguished, in most cases, though of course each gradually passes into the following.

Starting with the normal well-filled intestine as in Fig. 2, or Fig. 1, the first changes consist in decreasing distension, so that the individual branches become more clearly distinguishable. Somewhat later the distal portions of these branches disintegrate and form a longitudinal band of dark granular substance, which appears somewhat like a longitudinal canal on each side connecting with the lateral intestinal branches (Fig. 3, also the pharyngeal region in Fig. 6). Under high magnification, however, these longitudinal bands are clearly seen to be the débris of the disintegrated terminal regions of the branches. The lateral bands make their appearance first in the more terminal regions of the body and progress toward the middle regions as the ends of the branches undergo degeneration.

But a part of the products of degeneration

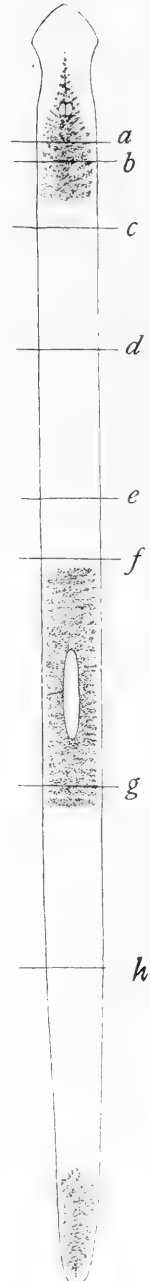


FIG. 1

appears within the intestine. As degeneration of the branches proceeds a fluid crowded with dark granular masses appears in the intestine and may accumulate and distend the remaining parts of the intestine in pieces of certain sorts to be described in another section. In normal animals, however, this substance never accumulates to any great extent but undergoes resorption almost as rapidly as it is formed and undoubtedly serves as nutritive material for other organs which are still functional.

As reduction continues the lateral bands become less conspicuous, the dark color gradually fading out as they undergo resorption, and the lateral branches undergo further reduction until their tips no longer extend to the region occupied by the lateral bands. At this stage the intestine appears as in Fig. 4 or as in the regions a short distance anterior and posterior to the pharynx in Fig. 6. Somewhat later still the lateral bands disappear entirely



FIGS. 2, 3, 4 AND 5

or break up into parts which sooner or later disappear. Various stages in the disappearance of the lateral bands are shown in Fig. 6. And finally, intestinal reduction may proceed so far that only the axial intestine remains (Fig. 5). In some cases, as in this figure, the intestine still shows slight indications of the positions of the former branches, but often even these disappear and absolutely no trace or indication of branches can be discovered (Fig. 19). Often, as in Fig. 5, the lateral branches disappear before the last traces of the lateral bands, which may persist for a time as isolated groups of granules, presumably occupying the parenchymal spaces originally filled by the ends of the lateral intestinal branches.

The next stage is of course complete disappearance of the intestine from the regions concerned. This stage is attained only in the terminal regions of the normal body and of pieces under certain conditions. In Fig. 6 the intestine has disappeared almost

entirely from the preganglionic region, in which it is present in normal well-fed animals (Fig. 1).

In all observed cases of intestinal degeneration, except under certain conditions connected with form-regulation, the course of the process of degeneration is essentially the same and passes through the stages described above.

## 2 *Discussion*

According to the above account the intestinal degeneration begins at the extreme peripheral regions of the intestine and proceeds "centripetally." The ends of the branches in the terminal regions are the first parts to disappear, and the last branches to undergo reduction are those immediately about the pharyngeal region.

It can scarcely be supposed that the more peripheral branches or the more peripheral regions of each branch are less needed than the more central parts and so disappear first. The peripheral portions of the intestine would seem to be just as essential as other parts for proper nutrition. The head-region is the most active region of the body and yet the anterior end of the intestine disappears earlier than any other part of the prepharyngeal intestine.

But when the course of reduction is considered from a functional standpoint interpretation becomes easy. In the first place the quantity of intestinal contents undergoes gradual decrease from the beginning to the end of the experiment. In well-fed animals the intestine is greatly distended (cf. Fig. 2) with food at first. This nutritive material is gradually used up, but as degeneration of the intestinal branches occurs a part of the products of degeneration appears in the intestine as a fluid crowded with dark granular masses. In normal animals this too undergoes resorption almost or quite as rapidly as it is formed, and gradually decreases in amount as time goes on. Thus even long after the food taken from without has disappeared the intestine is not empty, but the amount of intestinal contents is always decreasing. The movements of this dark substance in the intestine can be readily observed and the following statements regarding their relation to the general muscular contractions are the result of direct observation.

Under extreme conditions of intestinal distension with material all parts may be subjected to equal or nearly equal internal pressure but when decrease in the amount of intestinal contents occurs,

as is the case when the animals are kept without food, the energy of the mechanical conditions connected with the contents must decrease more rapidly in the peripheral than in the middle regions. Thus, for example, if the intestine is only partly filled, the internal pressure on the walls in the extreme anterior and posterior regions is in general much less than in regions nearer the middle. In the first place, the fluid contents are forced into this region only during extreme extension and then apparently with much less energy than into regions nearer the middle. The consequence is that those regions in which the functional stimulus falls below a certain minimum gradually undergo atrophy and degeneration, and as the intestinal contents continue to decrease in amount this atrophy and degeneration gradually extend toward the middle region, which is the last to be affected.

Size of the lumen of the various parts and friction between the contents and the walls must also play a part in determining movements and internal pressures of the intestinal contents and both of these factors tend to reduce the energy of the functional conditions more rapidly in the peripheral than in the middle regions.

The lateral intestinal branches in and about the pharyngeal region persist longer than any others, simply because the functional conditions are less altered there than elsewhere. In the first place, contraction which drives the intestinal contents toward the middle is usually sudden and violent, in consequence of sudden external stimuli, while extension is usually much slower and less extreme. Consequently the intestinal contents are driven into the lateral branches of the middle regions with great force long after they have ceased to reach the extreme peripheral regions at all. The normal movements of the animal, especially



FIG. 6

the very frequent slight contractions of the anterior and posterior end all tend to keep the middle regions of the intestine more distended than the peripheral regions.

Very probably the other functions, *i. e.*, the digestive and storage functions, also play a part in determining atrophy. Of course absence of intestinal contents from any part of the intestine means absence of food to be digested and stored up. Hence the cells of this region may atrophy or change their character because of the partial or total absence of the stimulus to the digestive function or because of malnutrition: or again degeneration may occur because the demands upon these cells for nutritive material are so great in relation to the supply, that they are exhausted or forced so far from equilibrium that continued existence is impossible: degeneration from either of these causes would affect the peripheral regions first and proceed toward the middle.

But as will appear below, in certain experimental cases it is impossible to account for the regulatory intestinal changes on any other basis than that of mechanical stimuli from the contents.

### III INTESTINAL REGULATION IN CORRELATION WITH FORM-REGULATION OF PIECES

The character of form-regulation in general in this species was described in an earlier paper (Child '05a). It will be recalled that regulation after removal of posterior pieces consists almost entirely in redifferentiation of the parts remaining, only a very small amount of new tissue being formed on the cut surface. Posterior regulation is qualitatively, *i. e.*, functionally, complete at all levels except anterior to, in, and immediately posterior to the cephalic ganglia. Regulation in the anterior direction, on the other hand, consists almost wholly of regeneration, except as regards certain cases of pharynx-formation, and is complete only at levels anterior to, in, and immediately posterior to the ganglia, being slight in amount elsewhere.

As might be expected from these differences, intestinal regulation is much more extensive in correlation with posterior than with anterior regulation. But the most remarkable cases of intestinal



regulation occur in cases where return to the typical form of the species does not occur.

In the earlier papers (Child '05a, '05b, '05c) dealing with this species the processes of form-regulation were interpreted as essentially cases of functional regulation, *i. e.*, "functional adaptation." For example the redifferentiation into a posterior end of the posterior part of a prepharyngeal piece and the formation of the pharynx at a certain level of the old tissue was regarded as the result of a functional regulation in response to altered conditions, in consequence of which a portion of the body which had been functionally, as well as morphologically, prepharyngeal now became functionally posterior, *i. e.*, postpharyngeal, and in consequence underwent regulation, *i. e.*, functional adaptation of its structures to the new conditions.

As I have pointed out repeatedly in different papers (Child '05a, '06a, '06b), redifferentiation of old parts into parts similar to those removed can occur only when these old parts are capable in some degree of becoming the functional representatives or substitutes of the parts removed. If functional substitution for the part removed does not occur at all, form-regulation does not occur: if the substitution is confined to regions adjoining the cut surface the part is replaced more or less completely by regeneration, the completeness of replacement depending on the degree of functional substitution.

As was shown in the earlier papers on *Cestoplane* (Child '05a, '05b, '05c), the phenomena of form-regulation in general can be readily and consistently interpreted on this basis and the differences between anterior and posterior, preganglionic and postganglionic regulation, and regulation in the presence and in the absence of the ganglia, differences which on any other basis appear merely as isolated facts without special significance and without relation to each other, are clearly correlated and explicable.

For the more complete discussion and interpretation of the experimental data in the light of this hypothesis the reader is referred to the earlier papers (Child '05a, '05b, '05c, '06a, '06b).

Since the phenomena of intestinal regulation are so striking in this species they were omitted from the preceding papers as deserv-

ing special consideration. As will appear, however, they afford strong support to the hypothesis which has served for interpretation of the other phenomena: indeed a consistent interpretation seems scarcely possible on any other basis than that of functional regulation.

## *1 Intestinal Regulation in Correlation with Posterior Form-Regulation*

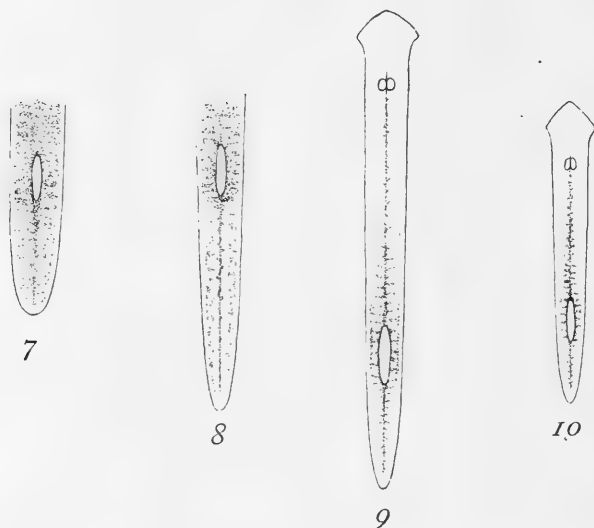
### *a In the Prepharyngeal Region*

The process of form-regulation in prepharyngeal pieces containing the cephalic ganglia consists essentially (Child '05a) in the redifferentiation of the posterior part into a new postpharyngeal region and the formation of a new pharynx between this and the new prepharyngeal region. Regeneration is limited to the extreme posterior end of the piece and amounts to little more than the closure of the wound. The length of the postpharyngeal region thus formed, and consequently the position of the new pharynx, depends on the level of the posterior end of the piece. If the piece includes only the most anterior part of the prepharyngeal region (Figs. 11 and 12), the new postpharyngeal region is short and the pharynx appears near the posterior end. With approach of the level of section to the original pharyngeal region the length of the new postpharyngeal region increases and the pharynx is formed farther from the posterior end (Figs. 7 to 9).

In all cases of regulation of prepharyngeal pieces containing the cephalic ganglia the lateral intestinal branches posterior to the new pharynx undergo complete disintegration within a short time after section, leaving only the axial intestine. This is shown in Figs. 7 and 8 for a long piece, and in Figs. 11 and 12 for a short piece.

In the first case the piece originally included that part of the body anterior to the line *f* in Fig. 1 and the new pharynx appeared at a considerable distance from the posterior end of the piece. During the first few days following section the dark color of the intestinal branches in the posterior part of the piece gradually fades. In six to eight days after section (Fig. 7), *i.e.*, after the

development of the new pharynx is well advanced, the intestinal branches posterior to the new pharynx are seen to be degenerating. The course of degeneration differs somewhat from that described above for normal animals. The branches appear broader and further apart as if this part of the intestine had been stretched longitudinally, and in all probability a mechanical elongation of this part does occur in consequence of its function as a posterior end and region of attachment. A few days later the branches disintegrate completely and the débris, appearing as dark masses



FIGS. 7, 8, 9 AND 10

and granules on either side of the slender axial intestine, gradually undergoes resorption until after two weeks or more (Fig. 8) scarcely any traces remain. A slender axial intestine still persists, however. The difference between the postpharyngeal region and the remainder of the body is striking (Fig. 8) for in other regions intestinal reduction has as yet scarcely begun. The sharp limitation of this peculiar process to the postpharyngeal region of the piece makes it certain that the disappearance of the lateral intestinal branches is correlated in some manner with the "redifferentiation" of this region from a prepharyngeal to a postpharyngeal region.

Intestinal reduction in other portions of the body goes on in the same manner as in normal animals, though somewhat more rapidly. But, meanwhile, short and slender new lateral branches develop on the postpharyngeal intestine in many cases. These never attained full development in the specimens observed, but there is no doubt that if the animals had been fed they would have developed and reached normal conditions. Fig. 9 shows the condition seventy days after section of the piece from which Figs. 7 and 8 were drawn. Intestinal reduction in the pharyngeal and prepharyngeal regions has followed the typical course but in the postpharyngeal region new branches have developed.

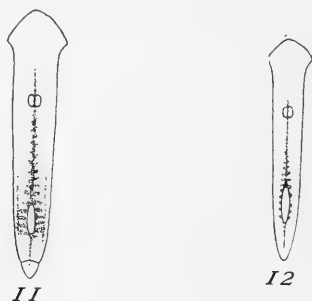
In still later stages without food reduction of all parts of the intestine takes place almost equally until only very short lateral branches remain (Fig. 10, 143 days after section). How much longer such pieces may live it is impossible to say, for my observations extended over only 143 days and many pieces were alive and active at the end of this time.

In shorter pieces the process is essentially the same. Taking, for example, a piece including that part of the body anterior to the line *b* in Fig. 1, the new postpharyngeal region is short, and the pharynx appears nearer the posterior end and the amount of regeneration is somewhat greater than in a long piece like the preceding. Fig. 11 shows this piece fifteen days after section. All traces of the postpharyngeal lateral intestinal branches have disappeared, only a very slender axial intestine remaining, which, however, extends a short distance into the regenerated tip. In the short pieces degeneration may begin in the redifferentiating region within four days after section, but in the long pieces does not usually appear for a week or more. As regards later stages a similar difference exists.

In these short pieces intestinal reduction in other regions is always much more rapid than in longer pieces. In Fig. 11, for example, a stage fifteen days after section, reduction is far advanced in the pharyngeal and prepharyngeal regions, and in Fig. 12, forty-five days after section, scarcely any traces of lateral branches exist in any part of the intestine. These short pieces usually die from forty to sixty days after section, *i. e.*, much

earlier than the longer pieces. In consequence of the more rapid intestinal reduction and earlier death of these short pieces lateral intestinal branches never develop in the postpharyngeal region.

These two pieces represent the two extremes as regards intestinal regulation in prepharyngeal pieces. The results in other pieces fall between these two extremes and differ in detail according to the part of the prepharyngeal region included in the piece. In every case, however, and my observations include some fifty cases, very rapid disintegration of the lateral intestinal branches took place in the region posterior to the new pharynx and in the larger pieces a new system of lateral branches developed later.



FIGS. 11 AND 12

In prepharyngeal pieces from which the head-region and the cephalic ganglia have been removed the formation of a new postpharyngeal region and pharynx takes place in the same manner as when the ganglia are present (Child '05c), the only difference being that the new postpharyngeal region is longer, the new pharynx farther from the posterior end and the process of degeneration somewhat less rapid than in pieces with posterior ends at the same level but containing the ganglia. As was pointed out in my earlier paper (Child '05c), the only ground which suggests itself for this difference is the functional relation between prepharyngeal and postpharyngeal regions. Removal of the ganglia reduces the functional activity of the prepharyngeal region very greatly, but affects the activity of the postpharyngeal region to a less extent, hence in regulation the reaction to the altered conditions at the posterior end involves more of the posterior region of the piece than in cases where the ganglia are present, since the energy of

reaction is greater in proportion to that of the prepharyngeal reaction, when the ganglia are absent, than when they are present.

In such prepharyngeal pieces without the ganglia the lateral intestinal branches in the region posterior to the new pharynx disappear in exactly the same manner as in the pieces already described, though apparently somewhat more slowly. The absence of the ganglia, therefore, does not affect intestinal regulation in these pieces, except somewhat as regards rapidity.

The products of degeneration of intestinal cells never accumulate to any great extent in these prepharyngeal pieces. Apparently they undergo resorption almost as fast as they are formed, serving, doubtless as nutritive material for the various regulatory processes, and all parts of the intestine become more and more slender and delicate as time goes on.

#### b In the Postpharyngeal Region

The character of intestinal regulation after removal of a part of the postpharyngeal region differs according to the relative length of the part removed. If the level of section lies only a short distance posterior to the old pharynx, *e. g.*, at the line *g*, Fig. 1, the intestinal changes which occur in the region posterior to the old pharynx are essentially identical in character with those described for prepharyngeal pieces and shown in Fig. 7 and 8. This region, originally the anterior end of the postpharyngeal region, redifferentiates into a whole postpharyngeal region, and the lateral intestinal branches disappear in the same manner as in pieces where the postpharyngeal region is formed from a part of the prepharyngeal region. One important difference exists, however; the degeneration is always less rapid in these than in prepharyngeal pieces, from three to four weeks being necessary for the disappearance of the branches.

Similar changes occur in pieces with posterior ends at levels somewhat posterior to *g* in Fig. 1, but with increasing length of the old postpharyngeal region in the piece, the degeneration of the lateral branches becomes slower and less complete, until, when half (*h*, Fig. 1) or more of the old postpharyngeal region remains, the lateral branches do not disappear early as in the

pieces described above, but simply undergo reduction as in normal animals.

### c Discussion

In the case of the formation of a new postpharyngeal region from a part of the old prepharyngeal region, or from the most anterior part of the old postpharyngeal region, all parts of the intestine except the longitudinal axial intestine degenerate completely in much less time than that required for reduction in normal animals.

These cases present certain peculiar features: here the intestinal material is present, but apparently for some reason the lateral branches are unable to persist in the region which undergoes redifferentiation. In later stages in the longer pieces small new intestinal branches usually develop from the axial intestine in the redifferentiated region. When the new postpharyngeal region is formed from the anterior half or more of the old postpharyngeal region, no such intestinal degeneration takes place.

How are these peculiar phenomena to be interpreted? The hypothesis that the intestinal material of the redifferentiating region is used up as nutrition for the growth of this region may serve to account for the rapid disappearance of the products of degeneration, but it does not serve to account for the degeneration of the lateral intestinal branches alone, while the axial intestine persists. Moreover, the process cannot be regarded in the light of an adaptation, for it is certainly not economical of material and energy, neither does it fit the animal better in any way for continued existence. On the contrary, it appears to be a useless destruction of structures of great importance, a waste of energy, and in every way a process which must result to the disadvantage of the animal.

But when we consider these cases from the functional standpoint they appear in an entirely different light. In the functional redifferentiation of a part of the prepharyngeal region into a whole postpharyngeal region certain changes in the mechanical conditions must occur. After such redifferentiation contraction of the body forces the intestinal contents in this region in the anterior

direction and extension in the posterior direction, whereas the reverse was originally the case. The intestinal contents now tend to enter the more anterior branches of the region during contraction and the more posterior during extension, but the branches were previously subjected to conditions the reverse of these. These altered conditions must bring about a very different distribution of the pressures and strains on the various parts of the intestine in this region. If the outline, arrangement and direction of the intestinal branches is determined in any marked degree by mechanical factors connected with the presence and movements of fluid contents, it seems impossible to doubt that such an extreme change in these factors must result either in a transformation of the original structures or in their disappearance, for they are the product of conditions the reverse of those now existing. Apparently the change is too great to permit transformation and the old structures disappear.

Moreover, if these mechanical conditions determine the intestinal changes in these cases, the persistence of the axial intestine is to be expected, for the functional conditions in it remain essentially as before, the direction of movement of the contents being merely reversed in each particular instance. Only slight quantitative changes, if any, are to be expected, therefore, in the axial intestine. As a matter of fact, the only change observed in the axial intestine in these pieces is a change in diameter in different regions. Instead of remaining larger as originally in case the piece was prepharyngeal, the posterior part becomes smaller than the anterior, a change which is doubtless correlated with the new functional conditions.

But the fact that the pieces in which the new postpharyngeal region redifferentiates from a short anterior portion of the old postpharyngeal region show the same rapid disappearance of the intestinal branches may perhaps be regarded as an objection to this hypothesis. It may be said that in these cases the mechanical conditions are not altered in the same manner and degree as in the prepharyngeal pieces and that the intestinal degeneration cannot, therefore, be due to such alteration. This objection cannot hold, however, as a moment's consideration will show. In these cases



a short anterior portion of the postpharyngeal region becomes functionally a *whole* postpharyngeal region and the change in mechanical conditions, although not a reversal as in prepharyngeal pieces, is without doubt great. The fact that when half or more of the original postpharyngeal region remains no degeneration, or practically none, except the usual slow process of reduction common to all specimens without food occurs, points in the same direction. The larger the part of the postpharyngeal region from which the new whole region is formed, the less the change in functional conditions associated with the functional regulation and the less the degeneration.

The facts as to rapidity of degeneration also support the functional hypothesis. The lateral intestinal branches of the redifferentiating region disappear most rapidly in short prepharyngeal pieces, where the new postpharyngeal region is formed from a region not far posterior to the cephalic ganglia. The rapidity of degeneration decreases as the level of the region from which the new postpharyngeal region is formed approaches the old pharynx. In pieces without the cephalic ganglia the rapidity of degeneration is somewhat less than in pieces with the ganglia. In those pieces in which the new postpharyngeal region redifferentiates from a short anterior portion of the old postpharyngeal region the disappearance of the intestinal branches is still less rapid than in the longer prepharyngeal pieces and, as noted above, in those cases where half or more of the old postpharyngeal region remains, the branches persist and undergo reduction in the usual manner.

It is not difficult to understand from the functional standpoint why these differences in rapidity of degeneration should occur. The change in the mechanical conditions in the intestine must be greatest when a region originally just posterior to the cephalic ganglia redifferentiates into a postpharyngeal region and least when the new posterior end is formed from a large part of the old postpharyngeal region. Between these two extremes the change is intermediate in degree. Evidently then the rapidity of degeneration in these cases is, as might be expected, parallel to the degree of change in the mechanical functional conditions. In the pieces without the ganglia movement is somewhat less energetic and less

frequent, hence the change in conditions in the region undergoing regulation is less extreme than when the ganglia are present, and degeneration is therefore somewhat less rapid than in pieces with ganglia. As will appear below, however, this is true only for headless prepharyngeal pieces of considerable length in which but little of the anterior end posterior to the ganglia has been removed. In short pieces neither a new postpharyngeal region nor a new pharynx is formed and the intestinal changes are very different from those described above.

The development of new short and slender intestinal branches in the postpharyngeal region after redifferentiation in the longer pieces is in all probability also a response to a functional stimulus. These branches correspond in arrangement and direction to the branches in a normal postpharyngeal region (Fig. 9). Their failure to appear in the shorter anterior prepharyngeal pieces is undoubtedly due to the fact that in these pieces the intestinal contents are used up more rapidly than in longer pieces, probably in consequence of the extreme activity which is characteristic of the short pieces: perhaps also the terminal region of the intestine contains less reserve material than other parts. Thus the intestine becomes almost completely empty and very thin-walled after about two months in pieces including only the anterior fourth of the prepharyngeal region, and the pieces die, while in pieces including the anterior three-fourths of this region this condition is not reached after about five months. Thus in the short pieces there is probably neither sufficient nutritive material available nor sufficient intestinal contents to furnish a stimulus to the formation of new intestinal branches in the redifferentiated postpharyngeal region.

It is of interest also to note that when new intestinal branches appear in the redifferentiated region they never develop to larger size than the intestinal branches of other regions which are undergoing reduction. It seems difficult to account for this early cessation of development on any other than a functional basis, but according to this hypothesis it is difficult to see why development should proceed farther, for the functional conditions connected with the presence of fluid contents are similar, quantitatively, in this region as elsewhere.

In short, when we consider the various features of this peculiar regulation as primarily "functional adaptations" or better as functional regulations, the morphological changes and results are readily interpreted. Moreover, I fail to see any other possible basis for interpretation. That additional factors may be involved, which have not been recognized, is extremely probable, but the facts themselves seem to me to indicate that mechanical conditions play a large part in determining the character of the functional regulation, which, in my opinion, is the basis of the morphological changes. Undoubtedly the process is, at least in large part, a complex physiological reaction, not a simple mechanical distortion.

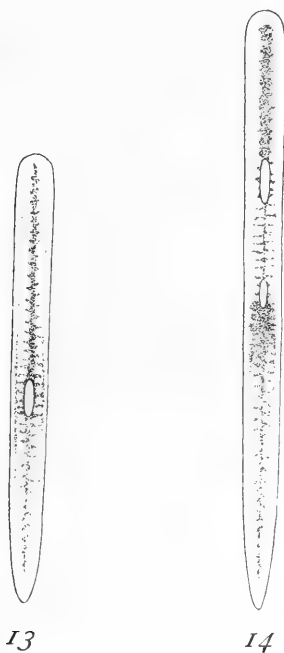
## *2 Intestinal Regulation in Correlation with Anterior Form-Regulation*

Anterior regulation is complete only at levels anterior to, through, and immediately posterior to the cephalic ganglia. The parts replaced are replaced chiefly by regeneration from the cut surface. In the case of regeneration of the head the ingrowth of the intestine into the new tissue requires no special consideration here, since it is similar to intestinal regeneration in various other species of turbellaria.

In certain cases, however, in which the anterior end has been removed posterior to the ganglia and no new head is formed, certain features of interest appear and these are considered briefly below.

Pieces from which the anterior end has been removed at a level not far posterior to the cephalic ganglia (*a*, Fig. 1) behave and react more like normal animals than pieces from which more of the anterior end has been removed: they are more active and react to slighter stimuli than the other headless pieces, but do not regenerate heads, although they produce more new tissue anteriorly than the others (Child '05a, '05c). In such pieces the only visible changes in the intestine consist in reduction of a type resembling that observed in normal animals. Fig. 13 shows a piece from which the anterior end was removed at a level corresponding to *a*, Fig. 1. The specimen was originally somewhat smaller than that

drawn in Fig. 1, so that the difference in size of the piece in Figs. 1 and 13 is not wholly due to reduction. Fig. 13 represents a stage 143 days after section. Comparison with Fig. 6, a normal specimen kept for about the same length of time without food shows that reduction is somewhat more advanced in the headless piece than in the normal animal. In such pieces, however, the axial intestine, especially in the prepharyngeal region, appears to be more or less distended by the dark colored products of degen-



FIGS. 13 AND 14

eration whose movements can readily be followed. This substance accumulates in headless pieces to a greater extent than in pieces with heads, undoubtedly because of the fact that these pieces, being less active than normal animals and pieces with heads, require less nutritive material and so do not use up the products of intestinal degeneration as rapidly as do the other pieces. Consequently the products accumulate in the intestine and, since the movements do not force the intestinal contents into the lateral

branches as frequently nor as strongly as in cases where the head is present, their effect appears chiefly in distension of the axial intestine. In the case of the pieces shown in Fig. 13 the prepharyngeal axial intestine is about the same diameter throughout its length, while the postpharyngeal axial intestine decreases in diameter posteriorly, because with the loss of the head the characteristic movements of the anterior end disappear to a large extent and conditions are much the same throughout its length, while the postpharyngeal region still exhibits the same regional functional differences as before, though its activity is somewhat decreased.

In cases where a somewhat longer portion of the prepharyngeal region is removed a second smaller pharynx appears (Child '05c). The pharynx varies in position according to the level of section. Where most of the prepharyngeal region remains it is usually a considerable distance posterior to the old pharynx, but in cases where most of the prepharyngeal region is removed it may be almost identical in position with the old pharynx and in such cases its formation involves the degeneration of the old pharynx. For the discussion of these cases in relation to functional regulation the reader is referred to my earlier paper (Child '05c). The point of importance for the present consideration is that a new pharyngeal region is formed in these cases and a new pharynx arises in it. But in many cases the old pharynx persists, at least for a time, so that conditions are different from those in other pieces.

In Fig. 14 a piece of this kind is shown at a stage 143 days after section. The level of section corresponds to *c* in Fig. 1. The old pharynx lies some distance anterior to the new and the intestine shows certain features of interest. Anterior to the old pharynx the axial intestine is large and much distended with the products of degeneration, but only short stumps of the lateral branches remain. Between the two pharynges, however, lateral branches are present, but both these and the axial intestine are very slender. For a short distance posterior to the second pharynx both axial intestine and lateral branches are large and filled with the dark substance, while in the more posterior regions they show the usual

features of reduction. The condition of the intestine and the visible movements of the intestinal contents in these and similar pieces in the later stages indicates that in the course of reduction in size the intestine sooner or later becomes occluded in the region of the pharynx or pharynges. During reduction in size the old pharynx is not reduced proportionally and in pieces which have been without food for several months it is often so large in proportion to other parts as to cause a bulging of the body-wall dorsally and ventrally in its region. It is probable that in such cases the pressure upon the intestine in the pharyngeal region is sufficient to prevent to a large extent the passage of intestinal contents through it. Similarly the development of a new pharynx, posterior to the old, as in Fig. 14, may likewise sooner or later occlude the slender axial intestine in this region. This being the case, the intestinal contents in the prepharyngeal and postpharyngeal regions do not enter the "interpharyngeal" region to any great extent, if at all. Consequently the course of intestinal regulation in this region is largely independent of that in other parts of the body. In the case shown in Fig. 14 this region contains but little fluid and both axial intestine and branches are slender, but since removal of the anterior end does not modify the muscular activities in this region except quantitatively to some extent, the branches have not entirely disappeared.

In the prepharyngeal region (Fig. 14), on the other hand, conditions are widely different. Here the axial intestine is greatly distended with a large amount of the dark substance. If Fig. 14 be compared with that portion of Fig. 1 posterior to the level *c*, which represents approximately the proportions of the piece at the time of section, it will be observed that the prepharyngeal region of the piece has decreased in size much more than the postpharyngeal region, doubtless, as was suggested in an earlier paper (Child '05c), because the energy of functional conditions in this region underwent a greater decrease with the loss of the head than in the postpharyngeal region, and so the former region has served in part as nutritive material for the latter. But the effect of this reduction in size on the intestine has been to hasten degeneration of the lateral branches in this region, since the movements of intes-

tinal contents have decreased in frequency and strength with the similar decrease in muscular activity. Moreover, the reduction in length of this region has resulted in confining the fluid contents which remain within a smaller space and so in filling this portion of the intestine more completely, since the products of degeneration form more rapidly than they undergo resorption. This pre-pharyngeal region of the body after removal of the head shows little differentiation of function, *i. e.*, the anterior end retains only in slight degree the characteristic motor reactions, hence the functional conditions are very similar throughout as regards the intestine, so that intestinal reduction shows no marked regional differences.

In the region posterior to the second pharynx, however, functional conditions remain much the same as in the normal animal (Child '05c), for the removal of the head affects the activities of the posterior end but little. Consequently contraction forces the intestinal contents anteriorly until they reach the region of the second pharynx, which they cannot pass, and so are forced into the lateral branches of this region and distend these. The second pharynx appears rather late and before its development the intestinal branches just posterior to it often undergo more or less reduction and after it appears enlarge again, very evidently in response to the altered functional conditions. The movements of the dark substance can be observed very clearly in this part of the intestine and the distension, accompanying contraction of the body, of the lateral branches just posterior to the second pharynx is very evident.

The fact that the products of degeneration accumulate in the intestine to a much greater extent in headless pieces than in normal animals and pieces with heads is a point of considerable interest. This accumulation cannot be simply the consequence of greater degeneration in these pieces, since in many cases it occurs in stages where degeneration is less advanced than in pieces with heads where no such accumulation exists. In normal animals and pieces with heads these products undoubtedly undergo more rapid resorption than in other pieces and are used to a greater or less extent as nutritive material for other parts, as has already been noted.

Their accumulation in headless pieces must be due to the fact that these pieces use the nutritive material less rapidly than those where the head is present. This is to be expected from the differences in activity between headless pieces and others. Moreover, it will be shown in the following section that these products accumulate more rapidly and to a greater extent in the intestine as the activity of the piece decreases. The absence of correlation between intestinal degeneration and the accumulation of the products of degeneration within the intestine indicates very clearly that the degeneration or persistence of the intestinal branches does not depend primarily on nutritive conditions. If movements are slight, intestinal degeneration may proceed more rapidly in pieces where a considerable quantity of the detritus, which undoubtedly possesses nutritive value, is present than in cases where the intestine is almost empty. Thus, for example, in the case just discussed (Fig. 14) the intestinal branches in the prepharyngeal region have undergone much more complete degeneration in 143 days than in a normal animal (Fig. 2), although the products of degeneration have accumulated in the headless piece to a much greater extent than in the other.

In this case then, as in those discussed above, the visible regulatory changes in the intestine are very evidently primarily functional regulations and are much more closely associated with the mechanical than with the nutritive conditions, *i.e.*, they are functional regulations in response to mechanical stimuli.

When a larger portion of the prepharyngeal region is removed, the second pharynx appears nearer the old pharynx, until in cases where the level of section is not far anterior to the old pharynx, this may persist, or it may degenerate and a small pharynx appear in approximately the same position (Child '05c). These cases present no new features of special importance as regards intestinal regulation. In some pieces the occlusion of the intestine by the pharynx or pharynges appears to be less complete than in others, and in such cases the peculiar conditions shown in Fig. 14 are less marked.

When the level of section lies immediately posterior to the old pharynx, a new pharynx is often formed at the anterior end of the



piece (Child '05c): in such cases the intestinal changes are essentially similar to, though more rapid than those in the postpharyngeal region of normal animals. Headless pieces entirely without a pharynx are discussed in the following section.

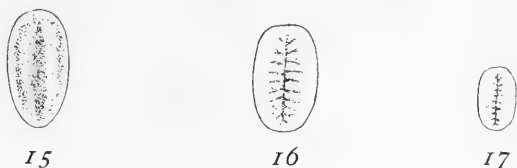
#### IV INTESTINAL REGULATION IN PIECES WITHOUT A PHARYNX

As was pointed out in an earlier paper (Child '05c), the isolated postpharyngeal region possesses the power of functional regulation only in slight degree. When the plane of section is immediately posterior to or near the old pharynx a new pharynx is often formed at the anterior end of the piece, but there is no visible redifferentiation of a part of the piece into a prepharyngeal region. In many such pieces, however, and in all postpharyngeal pieces in which the level of section is any considerable distance posterior to the old pharynx, a new pharynx does not appear, *i. e.*, these pieces do not possess sufficient power of functional regulation to give rise to any of the other regions of the body. The same is true of pieces below a certain length from the prepharyngeal region posterior to the ganglia. But these pieces, although they remain wholly without a pharynx and show practically no regeneration beyond wound-closure and no regulatory formation of other regions by redifferentiation, do present certain remarkable features as regards intestinal regulation.

Such pieces show few of the typical reactions (Child '05a, '05c): they do not usually attach themselves to the substratum, but are merely propelled through the water by their cilia: they rarely extend to full length and in course of time become greatly shortened and rounded and show almost no muscular activity beyond slight contractions and extensions and peristaltic waves which pass from one end of the body to the other. Many such pieces, however, were kept under observation during 143 days and the experiments were concluded only because of my departure from Naples.

Two such pieces are selected for description: all others observed are essentially similar. The first of these was a short prepharyngeal piece, including approximately the region between the levels

*d* and *e* in Fig. 1. Fig. 15 shows the piece twenty-six days after section. It is much reduced in size and degeneration of the intestinal branches has been very rapid. At this stage the axial intestine was distended by a large quantity of the products of degeneration. This rapid degeneration and the accumulation of the products of degeneration certainly cannot be interpreted as the result of lack of food. The intestine was well filled at the time of section and by no means all of its contents were lost through the wound; moreover, the motor activity of the piece is very slight and its need for food is therefore less than that of more active pieces; it has not formed extensive new parts either by redifferentiation or regeneration. If nutrition is the primary factor in determining the degeneration or persistence of the intestinal branches, we should certainly expect that they would degenerate very slowly in such pieces. Yet they degenerate more rapidly than in any other case except



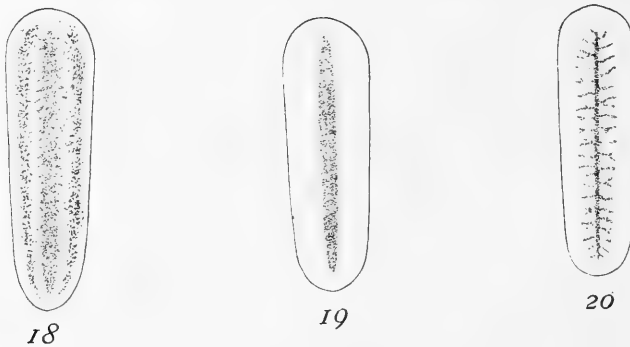
FIGS. 15, 16 AND 17

those in which a part of the prepharyngeal region redifferentiates into a postpharyngeal region.

About forty days after section no trace of the intestinal branches remained. If the experiment were carried no further, such pieces might be regarded as cases of "reversal of development," similar to those described by various authors as occurring during starvation. But after sixty-five days the piece presented the appearance shown in Fig. 16. A complete new set of very slender and delicate intestinal branches had developed and these persisted as long as the piece was kept under observation, but underwent gradual reduction as the size of the piece decreased in size (Fig. 17, 130 days).

The history of postpharyngeal pieces without a pharynx is essentially similar. Figs. 18 to 20 give three stages in the history of a piece corresponding to that portion of the body posterior to the

level *g* in Fig. 1. Fig. 18 shows the condition of the piece forty-five and Fig. 19, sixty-five days after section. At the latter stage all traces of intestinal branches have disappeared and the wall of the axial intestine, which is greatly distended with the products of degeneration, is as smooth as that of a rhabdocœl intestine. But forty days later, *i. e.*, 105 days after section (Fig. 20), new slender branches had developed along the whole length of the axial intestine, and these underwent gradual reduction, but were still visible when the experiment was concluded at 143 days. The development of the new intestinal branches in these pieces usually requires some twenty days or more. The various stages were examined with great care, in many cases under pressure, and there



FIGS. 18, 19 AND 20

is no possibility of error. Their development follows in every case the great distension of the axial intestine with the products of degeneration. Similar intestinal changes were observed in every piece incapable of forming a new pharynx, provided it did not die too early. In general the rapidity with which the changes occur increases with decrease in the length of the piece. This difference in rapidity is well shown in the two pieces selected for description. In the first, which is considerably shorter than the other, all traces of the intestinal branches were lost within forty days and the new branches were present after sixty-five days, while in the second, *i. e.*, the longer piece, the degeneration of the branches required sixty-five days and the development of the new branches, forty days more, in all 105 days.

In these pieces then, although there is no other visible regulation except wound-closure, the original intestinal branches undergo complete degeneration and a new set of shorter and more slender branches develops in their place. Moreover, the development of new intestinal branches occurs only after two or three months, during which time the pieces have undergone considerable decrease in size. Evidently it is not correlated with other processes of form-regulation in these pieces.

These remarkable phenomena seem to me to constitute a practical demonstration of the hypothesis which has served as the basis for interpretation of the other phenomena of intestinal regulation in this species. As a matter of fact they play an important part in the development of the hypothesis in my mind. A brief discussion will serve to show clearly their correlation with mechanical conditions.

In the first place muscular activity is relatively very slight in these pieces, consequently the movements of the intestinal contents are also relatively slight. Under these conditions the intestinal contents accumulate, as can readily be observed, in the axial intestine and enter the branches but little, except when more powerful contractions are induced by artificial stimulation. The old intestinal branches, not being adapted to these conditions, undergo very rapid degeneration and only the axial intestine remains. The persistence of this part of the intestine is to be expected, since all muscular contractions cause movements of its contents, and since these are accumulating as time goes on.

These pieces require little nutrition in consequence of their relatively slight activity, hence the products of degeneration do not undergo resorption as rapidly as they are formed, but accumulate in the intestine to such an extent that they distend it greatly, and finally bring about the formation of a new set of intestinal branches, which are adapted to the new conditions, and which undergo gradual reduction as these conditions change in following stages. Only in pieces where the axial intestine becomes distended with the products of degeneration do these new branches appear. In general, as the length of the piece decreases, the rapidity of degeneration of the old branches increases and the distension of the

axial intestine and the development of the new branches occur earlier. This difference in the rapidity of change is also exactly what might be expected according to our hypothesis, for the characteristic muscular activity and consequently the movements of the intestinal contents into the branches decrease as the length of the piece decreases, hence the shorter the piece, the greater the change in mechanical conditions affecting the intestinal branches. If the mechanical conditions are the determining factors, it follows that the rapidity of degeneration must increase with decreasing length of the piece. But increased rapidity of degeneration results in more rapid accumulation of the products of degeneration in the axial intestine and so in earlier development of the new branches.

This interpretation seems to me the only one possible. These cases show very clearly that the factors which determine the degeneration or the development of a structure are not necessarily associated primarily with nutrition or its absence. Development without energy is of course impossible and this energy must come from nutritive material of some sort. But the mere presence of the material does not necessarily determine that a given structure shall develop. That, as I have endeavored to show in most of the papers of this series and in others as well, is determined by functional conditions in the widest sense.

#### V THE RAPIDITY OF GENERAL INTESTINAL REDUCTION UNDER DIFFERENT CONDITIONS

Intestinal reduction in the whole body or piece proceeds with very different rapidity in different cases. The rapidity of reduction in certain special cases has already been discussed in the preceding sections, but a general comparison of the various cases presents certain features of interest since it shows very clearly that nutritive conditions are, at least in certain cases, not the only, nor even the most important, factors in determining the rate of intestinal reduction.

In the first place intestinal reduction proceeds more slowly in the normal animal without food than in headless pieces of any size. This is evident from a comparison of the figures. Fig. 6 repre-

sents the condition of a normal animal after about four and one-half months without food; Fig. 13 shows a piece including almost the whole body except the head after the same length of time without food. Shorter headless pieces differ still more widely from the normal animal; Fig. 15 shows a short, headless, prepharyngeal piece after twenty-six days of starvation and Fig. 18 a headless postpharyngeal piece without pharynx after sixty-five days. In these pieces total, or almost total, disappearance of the intestinal branches has occurred in a period of time from less than one-sixth to about one-half that necessary for intestinal reduction in the normal animal to the condition shown in Fig. 6.

Such differences as these cannot be due to differences in nutritive conditions. The normal animal is more active and must use a greater amount of nutritive material in proportion to its size in a given time than a headless piece such as that shown in Fig. 13, and its activity and nutritive requirements must be many times greater than those of the headless pieces shown in Figs. 15 to 17 and 18 to 20, in which movement is slight, yet in all of these pieces intestinal degeneration is more rapid than in the normal animal.

Moreover, in headless pieces the rapidity of intestinal reduction increases with decrease in the length of the piece. The long piece in Fig. 13 (all that part of the body posterior to *a* in Fig. 1) reaches the condition shown in 143 days, the piece shown in Figs. 18 to 20 (that part of the body posterior to *g* in Fig. 1) loses all traces of intestinal branches in sixty-five days, and the piece shown in Figs. 15 to 17 (that part of the body between *d* and *e* in Fig. 1) loses all traces of intestinal branches in less than forty days (Fig. 15, twenty-six days). In these pieces, and in all similar pieces observed, the rapidity of degeneration of intestinal branches is in general inversely proportional to the length of the piece.

At the time of section the amount of nutritive material in these various pieces must be about the same in proportion to their size. Of course some differences exist in this respect and there is more loss from the wound in some cases than in others, but contraction is usually so rapid that loss from the wound is slight. The two pieces whose history is given in Figs. 15 to 17 and Figs. 18 to 20 were taken from the same worm; nutritive conditions must there-

fore have been very similar in both at the time of section. But the muscular activity of the headless pieces decreases in general with decrease in length. The longer pieces must therefore use up nutritive supplies more rapidly than the shorter pieces and if degeneration were due to lack of nutrition it must occur earlier and proceed more rapidly in the longer than in the shorter pieces. But exactly the reverse is the case. Moreover, the formation of new intestinal branches several months after section and after the old branches have undergone complete degeneration shows very clearly that sufficient nutritive material is present to allow the development and maintenance of the intestinal branches, when the proper stimulus is present. It seems impossible, therefore, to escape the conclusion that nutritive factors are not the most important in determining the rapidity of intestinal degeneration.

But when we consider the dynamic conditions resulting from the presence and movements of the intestinal contents, it at once becomes evident that the rapidity of degeneration is in general proportional to the change in these conditions. In the normal animal these conditions remain most nearly normal and after the food taken from without has disappeared from the intestine it still contains a certain quantity of fluid, which moves about in the characteristic manner, though its effect must be quantitatively less than when the intestine is well filled. In headless pieces the movements differ more or less from those of the normal animal and are always less energetic and less frequent, hence the functional stimulus from the contents must be less than in normal animals and intestinal degeneration must occur more rapidly in such pieces than in normal animals if it is correlated with decrease or absence of these stimuli. Moreover, motor activity of all kinds decreases with decreasing length of the headless pieces and the mechanical stimuli arising from the intestinal contents must decrease similarly, especially in the lateral branches, since the less powerful the muscular contractions, the less frequently do the intestinal contents enter the branches. Consequently degeneration of the intestinal branches must occur with increasing rapidity as the length of the piece decreases, if it is connected with these conditions.

As shown above, the facts correspond exactly with the require-

ments of the hypothesis and I fail to see how any other interpretation of them is possible. They all indicate that the rapidity of degeneration of the intestinal branches is dependent, at least in large measure, on the degree of change in the mechanical functional conditions connected with the presence and movements of the intestinal contents, irrespective of their nutritive value.

But when we compare normal animals with pieces which possess heads the case is somewhat different. The very rapid intestinal degeneration in the redifferentiating regions of such pieces has already been discussed in Section III c, and does not concern, us here, but the rapidity of intestinal degeneration in other parts of the body differs from that in normal animals and also differs according to the length of the pieces. In such pieces the activity remains the same as in normal animals, or in short pieces including little besides the head-region, is apparently even greater than normal. Consequently these pieces must require as much nutritive material in proportion to their size as do normal animals, or probably even more in the case of short pieces. Moreover, these pieces undergo qualitatively complete form-regulation, producing a new postpharyngeal and pharyngeal region with a new pharynx. These changes must also require nutritive material. In such pieces the intestinal contents decrease rapidly in amount—the shorter the piece, the more rapid the decrease—and those portions of the intestine remaining never contain any considerable amount of the products of degeneration as do those of the shorter headless pieces. These products appear to undergo resorption almost as rapidly as they are formed. Consequently the quantity of intestinal contents becomes very small and the axial intestine and all other parts become very slender (Compare for example Figs. 9 and 10 with Figs. 15 and Figs. 18 and 19). As noted above this difference indicates that the products of degeneration serve as nutritive material. Since this does not accumulate to any extent in the pieces with heads the intestine becomes almost empty and, notwithstanding the normal movements of these pieces, the mechanical stimulation of the intestinal walls must be very slight and must decrease centrifugally. Consequently the branches disappear and the rapidity of degeneration is determined, at least in part, by the



rapidity with which the intestine is emptied of its contents in consequence of the demand for nutritive material. Therefore intestinal degeneration increases in rapidity with decreasing length of the pieces, as is the case in the headless pieces.

According to this interpretation the increasing rapidity of degeneration with decreasing length is due in headless pieces primarily to decreasing movement of the intestinal contents in consequence of decreasing muscular activity, while in pieces with heads it is due primarily to decreasing quantity of intestinal contents in consequence of the great demand for nutritive material. In the headless pieces nutritive material arising from the degeneration of the intestinal branches accumulates in the remaining portions of the intestine, but the branches disappear in spite of its presence. In the pieces with heads, on the other hand, this material is used up almost as rapidly as it is formed and the branches disappear because the intestine is nearly empty. In the first case the degeneration is apparently due largely to lack of movement of the intestinal contents, in the other to lack of intestinal contents to be moved.

Thus the data concerning the rapidity of intestinal degeneration serve still further to support and confirm the conclusion that intestinal regulation in this species is in large part a functional regulation in response to mechanical stimuli.

## VI CONCLUSION AND SUMMARY

The phenomena of intestinal regulation certainly afford strong support to a dynamic or functional hypothesis of regulation and in this respect are in accord with various other phenomena in this and other species, which I have described and discussed in previous papers. The intestine retains its typical form, or returns to it, only when dynamic conditions are, or become similar to those which give rise to the typical form. Extensive intestinal regulation may occur in the absence of other form-regulation, or intestinal regulation may fail to occur, while other parts undergo more or less complete regulation. The results in each case are correlated with the dynamic conditions in the intestine, particularly the mechani-

cal conditions, and can be interpreted only on the basis of this correlation. There can be little doubt that intestinal regulation in various other species of turbellaria will prove to be similarly dependent on mechanical conditions.

The history of the pieces without pharynges shows how little significance there is in description or discussion of "reversal of development" without consideration of the dynamic factors involved. When these dynamic factors act in reverse sequence and direction from that typical of normal development, then, and not otherwise, does reversal of development occur. There is no law, such as certain authors seem to postulate, that causes an organism to return more or less completely to an earlier stage of development if deprived of food, or under other changed conditions. The so-called "return" usually consists simply of the loss of previous differentiation, but this does not necessarily constitute a reversal, for the method of loss may be very different from the method of acquirement, as in the present case. Moreover, the loss of the original structure or differentiation may be merely the first step in the development of something new in response to altered conditions, as is the case in the pieces without pharynges. These pieces are in no sense returning to an earlier stage of development or "embryonic condition," because they lose the old intestinal branches, but are merely undergoing a process of functional adaptation or regulation. The gradual simplification in intestinal structure, which occurs in various planarians in the course of starvation and reduction in size, is undoubtedly essentially a functional regulation just as truly as is the appearance of new branches under other conditions.

Objection to my interpretation of the facts may perhaps be made on the ground that the recent experiments of Babák ('06) with amphibia indicate that chemical factors are much more important than mechanical in determining intestinal regulation. It can scarcely be doubted, however, that the amphibian intestine differs greatly from the turbellarian in function. As I have pointed out in Section I, the turbellarian intestine is much more than a digestive organ, being both a storage-reservoir for excess of undigested food-material and to a considerable extent a circulatory system. It

would be remarkable if mechanical factors were not much more important functionally in the turbellaria than in the amphibia. In order to interpret regulatory phenomena it is of the utmost importance to consider all the functions of an organ or structure and not merely one, or the most conspicuous. Only in this way shall we attain complete interpretation. It must be borne in mind that the name assigned to a part does not always indicate fully or exactly its functions, nor is the function commonly assigned to it necessarily its only function: in most cases it is merely a small part of the actual function.

In the present case the changes in mechanical conditions are to a certain extent visible and accessible to experimental methods and, as I have endeavored to show, the processes of regulation in the intestine are evidently closely correlated with them; indeed it is impossible to account in any other way for certain of the changes, such as the rapid degeneration in a postpharyngeal region formed by redifferentiation and the development of new intestinal branches in pieces without pharynges after months of starvation. Moreover, while other factors, such as the character of the food and the digestive activity, doubtless affect the structure of the cells and very probably their number, it is difficult to understand how factors of this kind alone can determine the form, arrangement and direction of intestinal branches. These elements of the intestinal form must, it seems to me, be determined mechanically, at least in large part, and it is with these that the present paper is primarily concerned.

The most important results are briefly stated in the following summary:

- 1 In normal animals kept for several months without food extensive intestinal degeneration occurs, beginning in the peripheral regions and proceeding toward the pharynx. This degeneration involves chiefly the lateral branches and affects the axial intestine only in the terminal regions.

- 2 In pieces undergoing regulation without food in which a postpharyngeal region is formed by redifferentiation from a part of the old prepharyngeal or the anterior part of the old postpharyngeal region, the old lateral branches of the intestine undergo rapid

and complete degeneration in the redifferentiating region and are replaced in the longer pieces by new branches, corresponding in arrangement with those of a normal postpharyngeal region.

3 In headless pieces which have not sufficient regulatory capacity to give rise to a new pharynx (short prepharyngeal pieces and most postpharyngeal pieces) the old intestinal branches undergo rapid and complete degeneration, but after two months or more a new set of short and slender intestinal branches arise, which persists, but undergoes gradual reduction as time goes on.

4 In all other pieces undergoing regulation without food intestinal reduction occurs and usually proceeds from the peripheral towards the middle regions, though special modifications occur with special conditions.

5 The intestine of polyclad and triclad turbellaria is not merely a digestive organ, but functions also as a reservoir for the temporary accumulation and storage of undigested food-material and also, to a considerable extent, as a circulatory system. Its contents are largely fluid and undergo movement in consequence of the muscular contractions of the body-wall. The presence and movements of these contents must produce characteristic mechanical effects upon the intestinal wall.

6 The facts of intestinal regulation indicate that these mechanical conditions play an important rôle in determining the outline of the intestine and the direction and arrangement of the branches. Total disappearance of the old branches occurs when the mechanical conditions are widely altered, even though nutritive material be present in excess. The rapidity of degeneration depends on the degree of change in the mechanical conditions. The development of new branches after degeneration of the old is determined primarily, not by the presence of nutrition, but by mechanical conditions, though of course nutritive material is necessary for such development.

Undoubtedly certain features of intestinal regulation are determined by other functional factors, but the general outline and the arrangement and direction of branches are very evidently closely correlated with mechanical factors.

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# THE BEHAVIOR OF LOXOPHYLLUM AND ITS RELATION TO REGENERATION

BY

S. J. HOLMES

WITH SEVEN FIGURES

## GENERAL CHARACTERISTICS OF THE SPECIES

The general form of *Loxophyllum meleagris*, the species studied, is flattened and leaf-like, and tapering toward the anterior end which is turned toward the dorsal margin. The anterior third or fourth of the body is flatter and less granular than the hinder portion and the margins of the body are thinned out, especially along the oral side. The middle and posterior regions are more convex and may be considerably distended when gorged with food. The body is ciliated on the right side on which the animal usually glides. The cilia are arranged in rows which extend in a longitudinal direction except near the anterior end of the body where they curve toward the dorsal side. The whole oral margin is also furnished with cilia, but none could be detected on the left side of the body.

The body wall is traversed with myonemes, both on the right and the left side, which extend longitudinally for the most part, but curve dorsally, like the rows of cilia, near the anterior end. They are more conspicuous and apparently thicker near the anterior end of the body, and they are especially well developed near the oral side. Trichocysts are abundant along the entire oral margin and around the anterior end of the body, forming a uniform series closely set at right angles to the surface. On the dorsal side the trichocysts are mainly confined to the small prominences, a dozen or more in number, which give that side its crenulated contour. Numerous trichocysts occur also on the right or ciliated side.

The contractile vacuole is nearly spherical in form and is situated near the dorsal side of the body a little in front of the posterior end. There is a fine canal extending from it anteriorly along almost the entire dorsal side. A short canal may lead into it from behind.

The meganucleus is composed of numerous rounded masses (over twenty in some individuals) scattered through the larger part of the body. The anterior third or fourth of the body, however, is usually free from nuclear material.

Although the mouth of *Loxophyllum* is an inconspicuous slit near the edge of the body the animal is nevertheless able to ingest comparatively large forms. Rotifers form a common article of diet. I have often seen *Loxophylla* containing specimens of *Anurea cochlearis* and other rotifers of as large size, the body being thereby much distorted in shape. In ejecting the lorica after the rotifer had been digested the body is much lacerated, but its power of rapid regeneration soon causes it to assume its normal outlines.

I have never been able to obtain *Loxophyllum* in abundance. Like many other predatory infusoria it thrives only in comparatively pure water and quickly disappears in the presence of putrefying material. It is found on aquatic vegetation, and sometimes appears on the walls of aquaria, especially those supplied with running water. A favorite situation is on the side of an aquarium just below the surface of the water.

#### NORMAL MOVEMENTS

The normal movements of *Loxophyllum*, compared with those of most infusoria, are sluggish, a circumstance which makes it easy to study the precise way in which they are performed. The creature glides along the substrate on its right side, moving its anterior end about slowly as if feeling its way. Its usual mode of locomotion is as follows: It elongates the body, swims forward a short distance, then contracts, swims backward, turns toward the oral side, and then elongates, and swims forward in a new direction. As it generally swims but a short distance before jerking back, the organism circles about toward the oral side in nearly the

same situation. I have often observed specimens on the side of an aquarium that remained over an hour within a few millimeters of their original position, although continually moving about.

It is an interesting fact that the motor reflex or avoiding reaction in *Loxophyllum* takes place in a direction just the reverse of that of *Paramecium* and many other infusoria; the turning is always to the oral instead of the aboral side. Most of the turning, however, occurs after the infusorian has ceased to swim backward which makes it probable that the anterior cilia are relatively more active at this time. While swimming forward the direction of movement is at the same time more or less toward the aboral side, and the backward movements are more or less toward the oral side, but the principal change of direction occurs at the close of each backward movement. The infusorian thus continually circles about to the oral side. When swimming backward the body is generally bent over to the oral side, often throwing the oral margin into one or more folds.

The movements of the body vary considerably in rapidity according to the degree of excitement of the animal, but I have never seen an individual in a state of absolute quiet. There is a certain regularity or rhythm of the forward and backward movements which is fairly constant for a long period. Most of the individuals in a dish move at a tolerably similar rate if some of them have not been more disturbed than others.

At times *Loxophyllum* may glide forward for a considerable distance without reversing its direction, but it does this, I believe, only when in a comparatively high degree of excitement. Its body is then strongly elongated and nearly straight. In the short forward movements which are performed in its usual circling about near one place the body is not so greatly elongated and it is bent over more strongly toward the aboral side. The extension and straightening of the body during its more rapid gliding aid the animal in maintaining a more direct course, although it commonly veers around somewhat to the aboral side.

The body of *Loxophyllum* is very mobile and it is able to change its shape in many ways by contracting locally in different regions. It may contract to half its maximum length, bend up or down or



to either side, or twist about on its long axis. It is almost constantly bending and writhing about in various ways. The anterior extremity is the most active as well as the most sensitive part of the organism. It is continually executing small movements, bending back and forth or up and down as if attempting to explore its environment. The oral margin of the body at times performs a sort of undulating movement, usually when it is lifted up free from the surface. This motion when the animal is largely free from contact with the substrate may become a vigorous and rapid one and serves to turn the body about in the water. When slight the fluttering movements are confined to near the anterior end of the body but when more decided they involve a considerable part of the oral margin.

When turned over so as to lie on its left or unciliated side *Loxophyllum* may right itself in several ways. At first it writhes about for a little while, but it is usually only a short time before one of its methods of turning over is hit upon. One common method is to raise up the ends of the body more or less, twisting about the anterior end until its right side touches the bottom. The rest of the body is then pulled over much as in the common righting movements of a planarian. Often, but not always, this is accompanied by a rapid undulation of the oral margin which apparently aids the turning. Generally *Loxophyllum* raises the oral side and twists about aborally, but it not infrequently turns over in just the reverse direction. Frequently the body is twisted about when the two ends are free in the water, the turning beginning at the anterior end and continuing until the whole body is twisted about.

When placed on its left side *Loxophyllum* sometimes bends the anterior end of its body upward at right angles to the long axis, raising it until it stands erect, and then toppling over upon the opposite side. In one instance I saw both ends raised up to about the same extent until they nearly met, forming a sort of hoop; then the animal rolled over, through the force of ciliary action until the anterior end touched the bottom, when it attached and glided ahead, thus straightening out the body into its normal position. *Loxophyllum* is, as a rule, rather reluctant to swim through the

water, but when in a state of unusual excitement it may do so quite readily. It swims in a spiral course like most infusoria, circling about in a clockwise manner and at the same time rotating on its long axis in the same direction. The spiral course is maintained not so much through the natural asymmetry of the body, as by the fact that the body is curved toward the inner side of the spiral and held at a slight twist. By means of its spiral movement *Loxophyllum* is able to travel in a nearly straight general course for a considerable distance.

#### REACTIONS TO STIMULI

*Mechanical.* In experimenting on the reactions of *Loxophyllum* to mechanical stimuli a glass rod was used which was drawn out into an exceedingly fine thread at the tip. By using a Braus-Drüner binocular microscope it was possible to apply stimuli of various degrees of intensity to any part of the body and readily observe the result. When the anterior part of the body is stimulated the animal contracts longitudinally, swims backward and to the oral side, and bends its body orally at the same time. After this it extends the body again and swims forward. Stimuli applied to the tip of the body most readily produce this reaction. It may be produced even without contact by moving the rod about a short distance in front of the anterior end.

Stimulating either side of the body back to a considerable distance produces the same reaction. It is obvious, therefore, that when the animal is stimulated on the oral side it turns directly toward the stimulus instead of away from it. Repeated applications of the stimulus to the oral side will cause the animal to keep turning toward the stimulus, notwithstanding the unadaptive, or even injurious, nature of the response.

The facility with which a stimulus evokes a response diminishes toward the posterior end of the body. Stimuli applied between the middle of the body and the posterior third, especially after the second or third trial, frequently produce no response, even when quite strong. The animal may often be poked about in this way, almost to the point of producing mutilation, without suffering any interruption of its usual activities.

If a stimulus is applied to the posterior end of the body, or a short distance in front of this on either side, the usual motor reflex is not produced. The animal swims directly forward. With repeated stimulation of the posterior end it may be kept swimming forward for a long time. If the stimulus is applied during the progress of the animal the rate of movement is accelerated. Essentially the same behavior has been found by Jennings to occur in *Paramecium* in response to weak stimuli, and I have often observed the same phenomenon in this and several other infusoria. It indicates the first step toward reacting in a specific manner to the localization of a stimulus.

The reactions of *Loxophyllum* are quickly modified by successive responses to stimulation. This, I believe, is in large part due to the dulling of the sensitiveness of the organism through the repetition of stimuli. A very slight stimulus to the anterior end of the body suffices at first to produce a reaction. With repeated poking the anterior end becomes so dulled that the organism may continue to swim forward in spite of frequent stimulation at this point. Recovery, however, is quick, for in a few minutes the responsiveness is as great as ever.

A similar result is more quickly reached by the application of stimuli to the sides. The motor reflex may be elicited very readily for a few times, but it soon requires much stronger stimuli to bring it about. If the stimuli are applied quite far back it requires fewer stimulations before the animal refuses to respond at all.

There seems to be a tendency for the organism to resume its usual activity which asserts itself when its sensitiveness becomes dulled so that it does not react so readily to stimuli. It may be kept from going forward, for instance, by repeatedly stimulating the anterior end of the body. But sooner or later the tendency to normal activity predominates and the animal may go forward in spite of considerable stimulation.

Notwithstanding its rapid habituation to stimulation *Loxophyllum* exhibits certain features of behavior that seem referable to the summation of stimuli. Repeated stimulation may induce a condition of unusual excitement which may be manifested in continual and quite rapid swimming, increased writhing movements,

or in increased rapidity of its ordinary back and forth movements. The reactions may be less easily evoked, but the spontaneous activity of the organism is increased.

*Chemical.* Owing to the small number of specimens available few experiments on the reactions of *Loxophyllum* to chemicals were tried, since they frequently produced fatal effects. Several drops of water containing specimens of *Loxophyllum* were spread out on a slide and a minute grain of salt or a small drop of weak acid was placed at one edge. The *Loxophylla* showed no tendency to go directly away from the diffusing chemical. Sometimes they would go toward it. In many cases they would move about irregularly until overcome by the chemical in case it was strong enough to be injurious. The majority of the individuals, however, usually succeeded, sooner or later, in getting away to a safe distance. When swimming toward the chemical the anterior end is more strongly stimulated and the animal swims backward, turns, and goes in some other direction. The length of the backward course being dependent on the strength of the stimulus received, the animal is apt to go back further when pointed toward the stimulus than when pointed away from it. Also excursions toward the stimulus are more quickly checked than those in other directions. In consequence of these reactions the animal works its way farther from the stimulating substance. The process is a slow one, especially since, owing to its natural rhythm of movement, *Loxophyllum* frequently changes the direction of its locomotion. Even when pointed directly away from the chemical it does not usually go very far before backing up and turning in another direction, and thus much of what was gained is lost. The whole process of negative chemotaxis in this form is a very slow, uncertain, and bungling one.

In one experiment I placed a lot of *Paramecia* with several *Loxophylla*, and a drop of acid was introduced at the edge of the liquid containing them. The *Paramecia* showed a very quick and marked negative reaction. The *Loxophylla* were incomparably longer in getting away from the chemical. Some went toward it and were killed, while practically all of the *Paramecia* got safely away from the injurious substance.

## THE BEHAVIOR OF PIECES OF LOXOPHYLLUM

It has been shown by Jennings and others that pieces of infusoria react in much the same way as the entire organism so far as this is rendered possible by the shape of the parts concerned. The observations which I have made on the behavior of pieces of *Loxophyllum* confirm the general results obtained by other observers and add a few points of interest, especially in relation to the subject of regeneration, which is treated in a subsequent section.

A specimen was cut transversely in two at about the anterior third. The two pieces swam rapidly apart, the anterior one going forward, the posterior one backward. For some time the anterior piece swam about in a circle toward the aboral side. After this it began to move alternately forward and aborally, and then backward and orally. At times the oral margin would be raised up and moved in an undulating manner like the edge of a flag, and sometimes the piece would turn completely over on its left side, but it usually glided along on its right side with little or no marginal motion. After about six minutes its backward and forward excursions became limited to about the length of its body. In going ahead there was a slight extension of the body, while in going backward the body was always widened. A little later its motions became confined to nearly the same spot. It would go forward, then backward, turning through twenty or thirty degrees, and then go forward again. Its behavior had become, therefore, much like that of the normal animal under usual conditions.

When the anterior end of the piece was stimulated by contact with a fine capillary glass rod it would swim backward and turn toward the oral side. When the posterior end of this piece was stimulated it would not react nearly so readily, and often quite strong stimuli produced no effect. When the response did occur, however, it was manifested in two different ways. At times the piece would flatten and swim backward, especially if the stimulus were strong. At other times the body would elongate and swim forward. In the first case it is probable that the animal was pushed ahead so that the more sensitive anterior end was stimu-

lated, and this would naturally produce a backward movement. The second response is like that of the normal animal when irritated at the posterior end. When the piece was swimming through the water stimulation of the posterior end frequently resulted in a marked acceleration of its speed.

The righting movements of the anterior piece were much like those of the entire animal. Generally the oral margin would be raised up and waved rapidly back and forth, a movement which probably causes the oral side to be elevated until the piece topples over upon its right side. Considerable variation occurs in the method of turning over in the pieces as in the whole organism.

In another experiment in which attention was paid mainly to the movements of the posterior piece a *Loxophyllum* was cut in two near the middle. The posterior piece swam backward quite rapidly for about three minutes. After this its movements became slower and it would swim forward occasionally. In a few minutes the forward movements began to increase, and after a while the infusorian settled down to moving forward and backward to about its own length. Sometimes it would raise itself from the bottom and tumble over on the other side only to quickly turn again into its normal position. At one time it left the bottom and swam in a spiral course for a considerable distance.

When moving backward the piece would widen out, especially at the anterior end. When moving forward, on the other hand, the piece would become elongated and strongly drawn together at the anterior end. These changes of shape were invariably associated with the different directions of movement.

Stimulation of the posterior end of the piece causes it to pinch together in front, elongate, and swim forward. When the anterior cut end is stimulated the piece spreads out and swims backward. Comparatively strong stimuli are required, however, in this case as the cut end is considerably less sensitive than other regions of the body. By stimulating either end of the piece it may be caused to swim continually forward or backward as the case may be.

In about thirty minutes after the cut was made the regeneration of the anterior end of the piece was well under way. The slight waving motions of the anterior border were visible, but not so

apparent as in the entire organism. The piece kept going forward and aborally a very short distance, then backward and orally, circling about in nearly the same spot, with a regular, incessant, rhythmical movement. In going forward the body became not only elongated but curved toward the aboral side. When contraction occurred during its reversed movement, it was greater on the oral than the aboral side as it is in the entire individual.

The behavior of several other pieces taken from the two ends of the body was essentially like those described. Sometimes the pieces would swim about in a spiral course through the water for several minutes, but eventually they all settled down to the same regular back and forth movements. Pieces cut from the middle of the body showed the same rhythmical movement, extending and bending slightly aborally as they went forward, and contracting more on the oral side as their motion was reversed.

#### RHYTHMICAL ACTIVITY OF LOXOPHYLLUM

When first observing the activities of *Loxophyllum* I came to the conclusion that the frequent reversals in the direction of its movements were due to reactions caused by minute objects with which the sensitive anterior end of the body came into contact. But further observation showed that these reversals were due to internal rather than external causes. When specimens of *Loxophyllum* were placed in water as free as possible from small particles the same regularity of reaction was found to continue. When gliding on the upper side of the surface film of a drop of clear water *Loxophyllum* reverses its movement about as often as when in the midst of objects with which it is continually colliding. But any doubt concerning the inherent rhythmicality of its movements is removed when we consider that the pieces into which the body is cut move back and forth at about the same rate as the whole animal.

The cut anterior ends of the pieces of *Loxophyllum* are comparatively insensitive to mechanical stimuli, and there can be no doubt that the movement of these pieces is a manifestation of rhythmic activity comparable to the beating of the heart muscle of higher animals. There seems to be no constant difference in

the rate of the back and forth movements between a piece containing the sensitive anterior end of the body and a piece from any other region. Even very small pieces show the same rhythm.

On account of its rhythmical activity *Loxophyllum* does not have to wait for something to turn up in order to acquire new experiences. Its life is one of continual trial. Only to a comparatively slight extent is its activity, under usual circumstances, directed by external conditions at all. It goes forward, back, turns orally, goes forward and back again, and so on, repeatedly, through its own inherent activity. In many of the lower organisms behavior mainly consists in more or less direct responses to external stimuli with little spontaneous movement, but unless something unusual affects it *Loxophyllum* keeps circling about near the same place for a long time. When it meets with a strong or injurious stimulus it has its own methods of getting out of the way, but its ordinary behavior is mainly guided by internally initiated impulses.

#### COMPLEXITY OF BEHAVIOR

From the preceding account it is evident that the behavior of *Loxophyllum* is considerably more varied than that of *Paramecium* and many other infusoria. *Paramecium*, for instance, has a very few stereotyped modes of behavior, such as spiral swimming, the motor reflex, acceleration of forward motion when lightly stimulated at the posterior end, the thigmotactic response, and bending the body when crowded among obstacles. *Loxophyllum* has not only all these responses, but several others in addition, *i. e.*, gliding movements, regular changes in the form of the body accompanying forward and backward movements, small feeling movements of the tip of the body, undulations of the oral margin, twistings, turnings, and contortions of the body under various conditions, special movements involved in swallowing large objects, and several kinds of righting movements. This greater complexity of behavior is probably a consequence of the fact that most of the creature's life is spent in contact with solid objects. It appears to be a general rule that the behavior of the



free swimming infusoria is more simple than that of the creeping or the permanently attached forms.

#### REGENERATION

While studying the behavior of pieces of *Loxophyllum* I found that regenerative changes set in soon after the animal was cut in two. A good opportunity was thus afforded for watching the regeneration of the animal which takes place so rapidly that one might almost be said to actually see it going on. To determine, so far as possible, the exact method followed in regeneration is always a matter of interest and importance; and a form in which

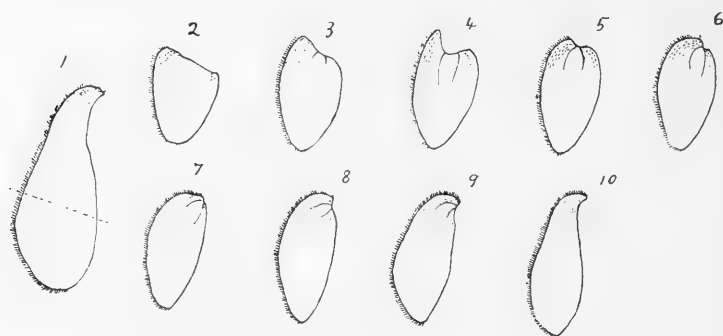


Fig. 1 Showing the course of regeneration of a piece from the posterior end of *Loxophyllum*. The dotted line in this and the following figures indicates where the cut was made.

the process can be watched under the microscope and followed step by step is especially favorable for this purpose.

A *Loxophyllum* was cut in two near the middle by a slightly oblique cut (Fig. 1). In the anterior piece the sides near the cut end were drawn inward and soon met, thus closing in the cut portion of the margin and giving the piece much the appearance, except in its relatively greater width, of the normal animal. In the posterior piece to which attention was mainly directed, since much greater modifications were necessary to restore the normal form, the first step in the process of regeneration was the closing in of the sides at the anterior end. The piece continued to swim forward and backward, undergoing the regular changes in form

that accompany these movements which have been previously described. Soon, apparently as a result of these stretching out movements that accompany its swimming forward, the piece began to acquire a more narrow and elongated form. With each forward motion the sides would be pushed around more toward the middle of the cut end which gradually became reduced in extent. With each movement ahead it could be seen that not only the body elongated but that it elongated more on the oral than the aboral side, causing it to bend toward the aboral side at each advance. This bend is not the result of the contraction of the aboral side, as one might very naturally suppose, but the exten-

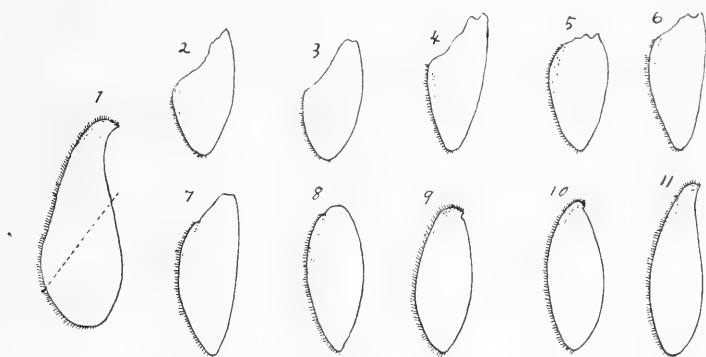


Fig. 2. Showing the regeneration of the posterior part of the body when cut off obliquely.

sion of the oral side. Soon the oral side begins to grow longer than the aboral and to become pushed around the anterior end of the body. The striations which originally ran in a longitudinal direction are now bent around the anterior end of the body more on the oral side than on the aboral. There is no formation of new tissue here, and no differentiation of new cilia on the cut surface, but the oral margin becomes stretched around the anterior end of the cut piece. Both sides of the body extend and contract, the movements being greater toward the anterior end. This end becomes (in consequence of these movements?) more narrowed and more like that of the normal individual. The cut end of the body is closed in by the gradual extension of the sides which fin-

ally meet, the point of union being carried by the greater extension of the oral side so that it finally comes to lie on the aboral side some distance behind the anterior end.

The method of regeneration here followed in restoring the external form of the body is the simplest and most direct that can readily be imagined. The elaboration of new structures is reduced to a minimum. The part of the infusorian behaves much as an entire individual, narrowing the body as it advances and stretching the oral more than the aboral side; and this behavior seems to help mold the part into the final form.

In order to find out how small a part of the differentiated oral margin could be stretched out to form the entire oral margin of the new individual a piece was cut obliquely across the body

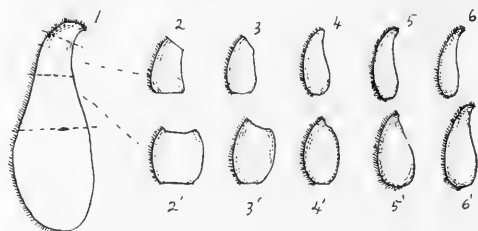


Fig. 3 Regeneration of small pieces from the anterior half.

(Fig. 2), so that the oral side of the posterior portion was considerably shorter than the aboral. In this case the general method of regeneration was much as before. Both sides curve in to close the cut end, the piece elongates and becomes narrowed; the oral side in the movements of the animal is extended more than the aboral, and we can see that it is gradually stretched forward; and finally it is pushed around the anterior end. The middle part of the cut surface which is becoming more and more reduced is apparently drawn back, but this appearance is due, I think, not to its being pulled back in the center, but to the extension of the two sides around it. The length of the ciliated oral side when regeneration is complete is considerably greater than at first. There was no extension of cilia in this case upon the cut surface. The anterior limit of the oral margin was very distinct and could

be followed in its course without any difficulty. The short oral margin of the posterior cut piece was simply stretched out to form the whole oral and anterior margins of the regenerated individual. Essentially the same method is followed in the regeneration of comparatively small transverse pieces (see Fig. 3).

The experiment was then tried of reducing the oral margin still more. By making a cut across the anterior end and a longitudinal cut near the oral side the whole oral margin was removed except a small part near the posterior end of the body (see Fig. 3). In this case the amount of cut surface exposed was very much greater than in the previous experiments, so much so that it seemed incred-

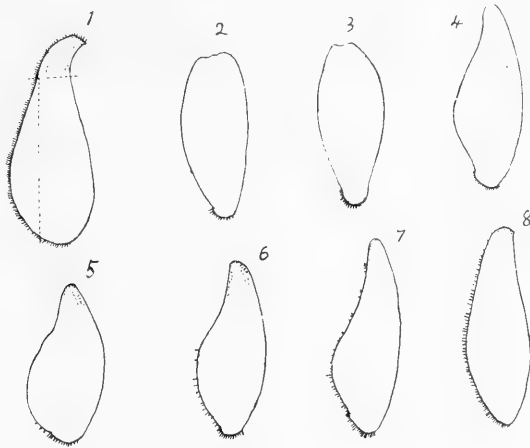


Fig. 4. Regeneration of a specimen from which the anterior end and most of the ciliated oral margin was cut off.

ible that the small remaining part of the ciliated margin could be extended so as to stretch over it, especially since the part remaining is one that gets little stretching during the usual activities of the animal. As might be expected, although it was stretched around the oral side to a certain extent, this part failed to give rise to any but a small part of the new oral side. The new oral margin with its differentiated structures had therefore to be produced by a new method. The piece began to elongate and become narrowed and rounded in front. Owing to the lack of the contractile and extensile elements of the oral margin the character-

istic pushing ahead of the oral side did not occur. The aboral side in fact began to be pushed ahead of the oral which accounts for the form of the pieces shown in the figures. After several hours the oral margin became thinner and clearer and the granules of the endoplasm came to lie further from the edge. A slight transverse striation could be detected in it such as occurs more plainly in the normal individual, and soon short cilia began to be put out here and there chiefly toward the posterior end. As the clear margin became broader it showed a longitudinal striation and soon began to extend and contract more during the movements of the body. As the oral margin slowly acquired its characteristic differentiation it began to push ahead and extend around the anterior end where its striations assumed the usual bend.

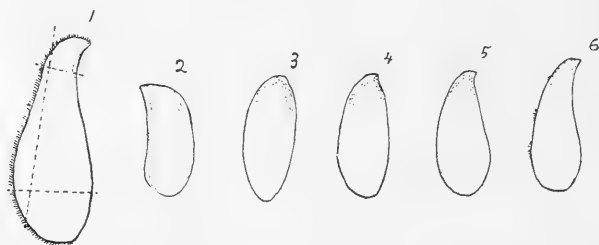


Fig. 5 Regeneration of a piece from the middle of the body from which the oral margin was removed.

In this case regeneration was very slow compared with the two preceding experiments. The piece was larger in size than the others but more differentiation had to be accomplished. Not until the oral margin became furnished with its cilia and its differentiated contractile elements so that it was capable of performing its usual rôle in the movements of the animal was there any marked progress in molding the body into its final shape. The anterior end, although it had become narrowed and rounded soon after the operation, did not take on any of its characteristic structural features until the oral margin became differentiated and began to be stretched around the front as in the cases of regeneration just described.

The development of the new cilia extended gradually forward from the small part of the ciliated margin that remained and the

possibility suggested itself that the new cilia which were developed arose through the influence of the old ciliated margin or of material which were developed arose through the influence of the old ciliated margin or of material which might be derived from it. To test this possibility a specimen was cut as is shown in Fig. 5 so as to leave no part of the ciliated oral margin remaining. The general course of regeneration is indicated by the Figs. 2-6. It will be seen that the aboral side, as before, extends at first more than the oral, but after the oral margin becomes differentiated in its characteristic fashion it pushes around more than the aboral and produces the usual curvature at the anterior end of the body. The cilia made their appearance in scattered groups about fourteen hours after the cut was made.

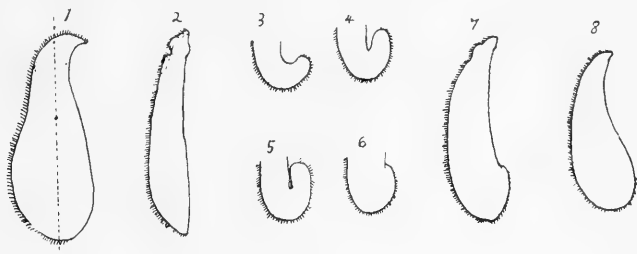


Fig. 6 Regeneration after removal of the dorsal half.

When *Loxophyllum* is cut in two longitudinally the process of regeneration is comparatively slow. The usual form of the body may be approximately reached in a comparatively short time, but the differentiation of the structures characteristic of either margin requires several hours. Fig. 6 represents a specimen from which the aboral half was removed by a longitudinal cut. The posterior end of the first became bent aborally and was brought forward so that the two parts of the cut margin met and fused together. The body as a whole shortened and widened; the injuries that were incidentally made near the anterior end of the body were repaired, and while the cut margin so far as could be ascertained seemed to close by the approximation of the upper and lower edges it was over twelve hours before the groups of trichocysts characteristic of the aboral margin made their appearance.

In a specimen cut longitudinally part way through the body (Fig. 7), regulation was effected by the meeting and fusion of the cut surfaces. The movements of this specimen were of interest. As it swam forward the two sides became crossed. During backward swimming on the other hand, they diverged very widely. This is doubtless due to the fact that in the lengthening and shortening that respectively accompany the forward and backward movements of the organism the marginal regions of the body are more active than the middle. The mechanism of the extension of the sides I have not ascertained.

#### THE RÔLE OF MOVEMENTS IN REGENERATION

The foregoing experiments make it probable, as Child has attempted to show in other forms, that the rôle of the movements

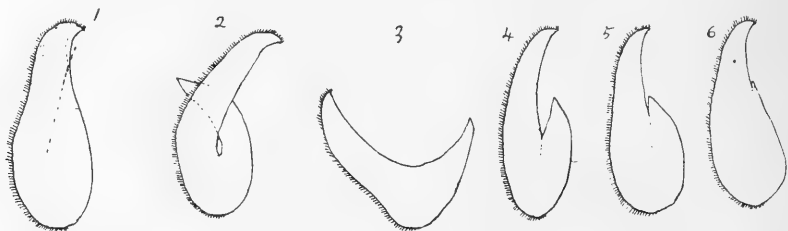


Fig. 7 Regulation in a specimen cut longitudinally as shown in 1. In 2 is shown the shape assumed when the animal is swimming forward; 3 shows the form during backward swimming.

of the organism in bringing about the normal form of the body is an important one. There are, in fact, few cases in which the efficacy of the factor of movements seems more manifest. To a considerable extent at least the organism seems to pull itself into shape. It has certain ways of acting which, as observations on the behavior of the parts have shown, are characteristic of the behavior of even quite small parts in much the same way as they are of the whole. A small piece cut from almost any region of the body shows the same rhythm of back and forth movements, the same correlation of extension with forward movement and of contraction with backward movement, and to a certain extent the same oral and aboral bendings as the entire animal. And when one carefully follows the course of regeneration it seems evident

that these movements are gradually working the part into the form of the whole. In the regeneration of the posterior half, for instance, one may see the oral margin extending and extending, growing a little longer with successive stretchings, until it curves about the anterior end of the body, and its striations are bent around so as to give the characteristic appearance of that region of the normal animal. The same kind of action is apparently instrumental in producing the same kind of form.

But precisely what is the relation of the movements of the organism to its regeneration does not, however, lie on the surface. It seems evident that the movements have an important part in shaping the general outline of the body. But are they the fundamental causes of this change of shape, or agencies which merely assist or accelerate the action of other formative factors? The experiments performed, while they indicate the importance of behavior in regeneration, show, I believe, that this factor is of a secondary or subordinate nature. It will be instructive to consider the course of regeneration in those experiments in which most or all of the oral margin was removed. Here regeneration was forced to follow a very different method from that adopted in the cases first described where a part of the oral margin was stretched out into the whole. The new margin had to be formed entirely *de novo*. There were involved the thinning out and clearing up of the oral side, the differentiation of new contractile threads, new trichocysts, new cilia, a complicated ordering of newly differentiating structures. The gross movements of the body could have had very little to do with all this. Until these differentiations were made the movements of this side of the body were not of the usual kind. Commonly the oral side extends and contracts more than the aboral, but when the marginal elements of this side were removed the opposite side was the more active. The oral side did not extend so rapidly as the aboral until the structures characteristic of the oral margin were established.

If in the experiments first described the general form of the body seemed to be produced by the characteristic behavior of the animal, the characteristic behavior in this case had to wait until its structural basis was established by comparatively slow differ-



entiations of new parts. If form seems in some cases to be molded by function, function in turn is apparently the result of organization. The modifications of form and function, of course, go on *pari passu*, and are after all but different aspects of the same process. The gross activities of the organism are largely dependent on the finer organization of the animal since they are carried on in a very similar way, even by comparatively small pieces of the body. Where, as in the experiment cited, the animal is cut in such a way as to modify certain of its grosser movements the finer differentiations go on until a structure is produced which then undergoes the movements characteristic of the whole when the external shape is rapidly assumed.

The processes of building up the finer structures of the body, the formation of new myonemes, cilia, etc., are really the fundamental features of regeneration. Pulling the body into shape is a sort of secondary matter in which the gross movements play an important part, to be sure, but these are themselves dependent upon the finer differentiations. In certain cases among the infusoria, such as some of the Hypotricha, the comparative rigidity of the body excludes the factor of movement from playing a very important rôle in shaping the outlines of the regenerating organism: Yet these forms regenerate with great readiness. Where the factor of movement is of importance in the regeneration of the infusoria, it is, I believe, rather in the nature of an aid to other formative factors than an essential and fundamental factor itself.

# REGENERATION AS FUNCTIONAL ADJUSTMENT

BY

S. J. HOLMES

WITH ONE FIGURE

In a previous paper<sup>1</sup> I have ventured to outline a general theory of form regulation, based on the conception of an essentially symbiotic relation between the parts of an organism. The conception is, of course, nothing new, but, so far as I am aware, no one has hitherto attempted to deduce from it a theory of regeneration and other processes of a regulatory nature. The theory may be stated in brief as follows: The various parts of an organism are supposed to stand in such a relation to each other that each part derives some advantage or is helped to perform its normal functions through the materials and stimuli it receives from other and especially the contiguous parts of the organism. Each part in turn contributes something to the normal functioning of the parts surrounding it; the relation is one of mutual dependence. Being mutually dependent, the parts of an organism tend to settle into a condition of functional equilibrium. When a part of the organism is removed and tissue of an undifferentiated nature is produced in its place, this new tissue develops in the direction of the missing part because this line of development is favored through the influence of the surrounding parts. Whatever advantages accrued to the part formerly in this position from its relations to the parts around it would also accrue to this tissue in so far as it differentiates in the same way as the part removed. The new tissue differentiates according to the functional demands upon it and its line of specialization may be regarded as a case of functional hypertrophy. Regeneration of the missing parts, therefore, may be interpreted as an expression and act of getting back into a condition of functional balance.

<sup>1</sup> Archiv für Entwicklungsmechanik, xvii, Bd., p. 265, 1904.

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The process might be illustrated by the case of a social organism composed of animal cells and symbiotic algæ which I described in my former paper. "We may suppose that both animal and plant cells tend to grow and multiply as far as circumstances permit. As these cells depend upon each other to a certain extent, neither kind of cell will tend to preponderate over the other, but they will all adjust themselves to a condition of approximate equilibrium. Now suppose that a considerable number of the algæ of this composite organism be removed. There is a functional demand by the rest of the organism for the products of the algæ and an excess food supply for those which remain. The algæ, therefore, are supplied with exceptionally favorable conditions for growth and multiplication, and will be stimulated to regenerate their missing number. By supplying the functional demand of the animal cells they indirectly benefit themselves, because by producing more oxygen they enable the animal cells to produce more of the substances which they utilize as food. If we suppose that in our hypothetical organism there are, in addition to the two kinds of cell mentioned, indifferent cells which are able to develop into either animal cells or algæ, it seems probable that, in the event of the removal of the algæ, the indifferent cells will differentiate so as to take the place of the missing numbers. \* \* \*

"For the sake of a simple illustration we have described an organism consisting of but two kinds of cells, but there is no reason to doubt that in a complex organism consisting of many varieties of cells standing in a symbiotic relation there would be a similar regeneration of any part that is removed. Let us imagine an organism made up of a number of differentiated cells, each of which derives some advantage from some substances produced by the contiguous cells, and giving out some substance upon which the contiguous cells are more or less dependent. We will suppose that, in addition to these differentiated cells, there are scattered through the body numerous indifferent or embryonic cells whose multiplication is held in check by the others, but which upon the removal of any part respond to the functional disturbance by growth and multiplication near the place of mutilation. We may represent our hypothetical organism graphically by the following

diagram in which the differentiated cells are represented by the larger circles *A*, *B*, *C*, etc., and the indifferent cells by the smaller circles between them. Each cell such as *A* contributes something utilized by *B*, *G* and *F*, and derives something in return from each of these sources. Now suppose *A* is removed; the indifferent cell lying near by, no longer held in check by the same stimuli, begins to grow and develop. What line of differentiation will it most naturally take. Owing to the symbiotic relation subsisting between the cells differentiation in the direction of *A* will be most favored as this secures it the advantages which *A* received. In other words, this will be the direction of development along which social pressure will tend to guide it. And the result will be a re-

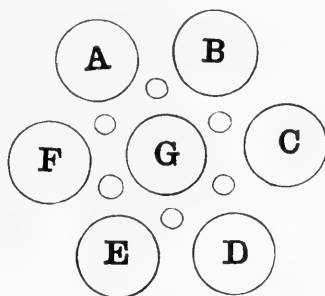


FIG. 1

generation of the missing part." For applications of this theory here set forth in barest outline to morphallaxis, heteromorphosis, physiological regeneration, and other modes of regulation, reference may be made to my former paper.

In some recent articles the problem of regulation has been approached from points of view somewhat similar to my own. Jennings<sup>2</sup> has attempted to show that regulation in behavior is fundamentally similar to other forms of regulation. The method of trial and error, which is so pronounced a feature of the behavior of lower organisms, and one through which they secure a large part of their adaptations to external conditions, is assumed by Jennings to be followed in the various processes occurring in

<sup>2</sup> This Journal, vol. ii, and Behavior of the Lower Organisms. New York, 1906.

the regulation of organic form. "A disturbance of the physiological processes," he says, "results in varied growth activities. Some of these will relieve the disturbance; the variations then cease and the processes are continued." The result of the selection of those growth processes which relieve the disturbance—or we might say make for functional equilibration—is the restoration of the lost part. Jennings has not attempted to develop a theory of form regulation in detail farther than to show the fundamental similarity of the method of regulation in various fields, but he holds that my own point of view so far as form regulation is concerned is in "essential agreement" with his.

Child<sup>3</sup> has recently outlined a theory of regulation which, as he states, is "somewhat similar to that adopted by" myself, although differing in certain important particulars. According to both Child and myself, regeneration and other formative processes are the result of functional activity, or more specifically, functional equilibration. Tissue differentiates in the direction of the missing part because it takes on the functional activity of the missing part. To cite an illustration by Child, "after removal of the anterior or posterior end in *Bipalium* or *Planaria maculata* the terminal regions of the piece remaining are subjected to conditions somewhat similar to those existing in the terminal regions of the part removed. The anterior end of the headless piece of *Planaria* is subjected to external conditions more or less similar to those to which the old head was subjected; moreover, its relation to the other parts of the body is more or less like that of the head. Stimuli resulting from forward movement affect it first, and are transmitted from it to other parts, etc. Functionally speaking, it serves in some degree as a head. The case is similar as regards the posterior end. After removal of the original posterior end, the posterior region of the piece functions in some degree as a posterior end, or to put the matter more strictly, its functional relations with other parts are more or less similar to those of a 'tail' or posterior end. \* \* \*

"Redifferentiation occurs as a result of a functional substi-

<sup>3</sup>Archiv für Entwicklungsmechanik, xx Band, 1906; and this Journal, vol. iii, 1906.

tution of a larger or smaller part of the old tissues of the piece for the part removed; the substitution may be imperfect or incomplete at first, and gradually attain completeness. In consequence of this functional substitution, the structure of the part involved is altered until it comes to resemble more or less closely that of the part removed."

Where regeneration through the formation and differentiation of new tissue occurs, it is this tissue which becomes the functional representative of the old part. In the regeneration, for instance, of the arm of a star-fish, or the leg of an arthropod or amphibian, the new tissue "must be subjected to many conditions—internal and sometimes external—similar in a greater or less degree to those to which the part removed or some portion of it was subjected."

The factor of the exercise of a part which Child has regarded in many cases of so much importance, is not always necessary for regeneration. "The growth of the new leg is not the result of the attempt to use the leg which is missing. The growing tissue begins to develop into a leg because its relations to the other parts of the system are in some degree similar to those of the leg removed. As it grows, the conditions approach much more and more nearly those to which the normal leg is subjected, *i.e.*, there is a gradual return of the functional conditions to the normal."

The application of Child's theory to the subject of polarity, heteromorphosis, and many other regulatory phenomena, it will not be necessary for our present purpose, to discuss. The fundamental idea of the theory is that form regulation is a result of functional regulation.

In certain cases Child attempts to show that this supposed functional relation actually occurs as a consequence of mechanical conditions. The anterior end of a planarian gets stimulated by the water and by the impact from foreign bodies much like the head does. The posterior end of *Stichostemma* is used by the animal in locomotion much as the tail is. At first the external stimuli affecting the ends are much the same whether they are anterior or posterior. In the movements of the animal both receive frequent impact from contact with various objects. There

is a difference perhaps in the mechanical stimuli received, but granting that these start the course of differentiation in different directions, they are entirely inadequate to account for the whole process of differentiation, as Child himself would probably admit. Where the factor of movement is absent, Child has recourse to the supposition of some other form of functional substitution, but he gives no clear account of why the substitution should occur. To say that the end of an arthropod's appendage is regenerated because of the functional activities that occur within it, that morphallaxis occurs when the part readily takes on the function of the whole, and that regeneration takes place because the functions of the missing part are imposed upon the new tissue that is developed in its place, may all be very true so far as it goes, but until some principle for the explanation of this functional adjustment is brought forward the explanation of regeneration is far from complete. If form regulation is a consequence of functional regulation, as Child and I agree that it is, the interpretation of functional regulation is the next obvious step. The inadequacy of Child's theory is, that it does not contain any general principle of explanation for that functional substitution and equilibration upon which it is assumed that form regulation depends. This, however, is a matter of incompleteness rather than error. But I suspect that when his theory comes to be developed so as to supply this missing element, it will involve, to make it workable, the assumption of some such symbiotic relation between the parts of an organism as I have assumed. It is a strong point in favor of the theory of symbiosis that it affords to a certain degree an explanation of physiological adjustment; in fact, it is primarily a theory of physiological equilibration. This physiological adjustment brought about through the symbiotic relations of the parts may, as I have attempted to show, be explained, or at least much of it may be explained, as the outcome of a tendency toward chemical equilibration. To the extent that this is true, we have an explanation of the regulatory activities of an organism in terms of familiar chemical phenomena.

The conception of something like a symbiotic relation between the parts of an organism which is involved in my own theory

of regulation, Child rejects, but I think on insufficient grounds. I have assumed that, according to the symbiotic relation of the parts of an organism, upon removal of a part, such as *A*, in the figure, the undifferentiated tissue in the region of *A* will differentiate in the direction of the missing part because of the functional demands, or for what, for want of a better term, I have called social pressure, upon that tissue. "This," according to Child, in referring to the particular case illustrated by the diagram, "is exactly what will not occur under these conditions. If all the cells *A-F* are symbiotically correlated then removal of one of them, *A*, must affect all the others, *i. e.*, the whole complex is altered by removal of one of its members. It is perfectly clear that the 'social pressure' of the altered complex will not be in the direction of differentiation of the indifferent cell into something like *A* but in some other direction, in other words, the indifferent cell cannot replace *A* but will form something different. Moreover, since all the cells were dependent upon *A* in some degree, the removal of *A* will probably render continued existence impossible for some of them and their place will be taken by the undifferentiated cells, but these will also develop into something different because the 'social pressure' is altered. It is perfectly evident that no regulation in the sense of replacement of a missing part could occur in such a complex."

Now this conclusion may be perfectly clear to Dr. Child, but I must confess—perhaps I am blinded by my bias in this matter—that it is far from being so to me. According to Child, since the removal of *A* would alter *B*, *G*, *F*, etc., not only something different would be developed in place of *A*, but the whole complex, according to my theory, would be profoundly altered. Now, I admit that the removal of *A* tends to alter *B*, *G* and *F*, etc. How far this tendency will result in a modification of these cells depends on the plasticity of the organism and the degree of mutual dependence of the parts—factors of course which vary in different organisms. But Child overlooks the fact that according to the symbiotic relation assumed, the other cells *C*, *D*, *E*, etc., tend to keep *B*, *F*, *G* in their original condition. In so far as these remain in their original state, their influence on the indifferent tissues in the



region of *A* will tend to mold it in the direction of the missing parts. In so far as *B*, *G* and *F* are modified through the loss of the missing part, their influence on the tissue in the region of *A* will come to be modified, and they will, in turn, modify the cells lying next to them. But, as there is a tendency for the modification produced by the loss of *A*, to spread successively to other parts, there is also a tendency, according to my theory, toward the checking and reversal of this process. If the loss of *A* tends to modify *B*, *F* and *G*, the presence of *E*, *C* and *D* tends to hold them in place, and in so far as these are maintained through this influence they tend to mold the tissue in the position of *A* into the form of the missing part; and in so far as this is so molded its modifying influence on *B*, *F* and *G* is diminished. How the process works out depends naturally on the degree of specification of the parts, whether or not new tissue is formed in the place of the missing part, and perhaps other factors. If the organism is plastic and its parts have not acquired an irretrievable set which prevents further modification, it may be entirely worked over in consequence of the disturbance of its social pressure in the vicinity of the missing part, thus leading to redifferentiation, or morphallaxis. Whether we have morphallaxis or regeneration in a narrower sense may depend, among other things, upon the degree of specification of the parts. As I have suggested in my former paper (p. 288), and as Child has maintained more at length, regeneration as opposed to "redifferentiation, increases as functional specification of the tissues increases or, in other words, the greater the degree of differentiation—the visible result of functional specification—the less likely is extensive functional substitution and consequent redifferentiation."

This, it seems to me, is very much what one might expect according to the theory of regeneration I have outlined. Replacement of *A*, according to Child, "can occur only when the relation is largely one-sided, *i.e.*, when *A* is dependent on *B-F*, but these latter are not to any marked degree dependent on *A*. In this case, and in this case only, will the social pressure force the undifferentiated cell to differentiate into something like *A*." Where redifferentiation from new tissue is concerned, as in the present

case, it is not the relation of  $A$  to  $B-F$ , that should be more or less one-sided, but the relation of the tissue in place of  $A$  to this complex. This is an important distinction which Child does not seem to have considered.  $B-F$  are relatively fixed, the tissue in place of  $A$  is young and plastic, and more dependent so far as the direction of its differentiation is concerned, upon  $B-F$ , than these are upon it. We may grant that, when regeneration occurs, the relation of dependence between the old parts and the new tissue is more or less one-sided, although the relations of the part removed may not have been. This would naturally result if the parts were relatively stable. They may be in a symbiotic relation, nevertheless, each part contributing in some way to the normal functioning of the others, and dependent to the extent that the removal of one part may alter only to a certain degree the quality and quantity of the activity of the surrounding parts, without producing extensive modifications of structure or function.

If the parts  $B-F$  were more plastic, absence of  $A$  would naturally tend to cause greater changes in them, especially if new tissue were not produced in place of  $A$ , which would come to assume some of the missing functions before the modification extended very far. There would be a progressive modification extending from the region of  $A$ , which would tend to become less the farther it extended, but eventually perhaps affecting more or less the entire organism. Functional equilibrium would then be maintained by working over the organism so that all the parts were adjusted to functioning on a smaller scale. The different methods of regulation, through morphallaxis, regeneration and the various combinations of these processes are, I believe, interpretable according to the symbiotic theory, and the relations of regeneration and morphallaxis to the degree of specialization of the parts which Child has elaborated, are, in fact, exactly what the theory would lead us to expect.

The difficulty pointed out by Child that the process of differentiation of a new part seems to begin at the tip and work back to the base is one which gave me some concern when developing my theory, but I think the difficulty is by no means a fatal one. When a developing limb shows first those structures character-

istic of its distal end we should bear in mind the possibility that the differentiation which first appears is not necessarily that which first occurs. What we know of developmental processes renders it very probable that a great deal of differentiation is going on in the rudiment of the limb before it is manifested by any external signs. Between maturation and cleavage an ovum may show no outward sign of differentiation, but experiment shows that this period is one in which developmental processes are rapidly taking place. Before any external features are produced in the development of a limb, the main outlines of its differentiation may have been established through influences proceeding from its basal part, after which the tip might differentiate more rapidly than the intervening portion, and the other visible features of structure appear successively toward the base. I do not suggest this merely to save my contention by a retreat into the invisible, but there are certain considerations that make such an interpretation more or less probable. In many cases the visible differentiation is centrifugal rather than centripetal. The tail of a tadpole cannot be said to differentiate from the tip toward the body as it regenerates. Zeleny has shown that in the early regeneration, as in the embryonic development of the antennæ of *Mancasellus* the formation of segments proceeds at first from the base to the tip; later new segments are formed in the reverse direction.

But granting that, in many cases, differentiation actually begins at the extremity and works toward the base of the regenerating organ, the process is not inconsistent with the point of view here set forth. We may suppose that the influence of the environment causes the extremity of an organ to begin to differentiate like that of the missing part. That is only one step. We have then to account for the numerous coördinated differentiations that take place as the part develops toward the base. In my illustrations of the course of differentiation under the guidance of social pressure, I have taken the old part as a starting point, but if we have an undifferentiated mass of cells, it is conceivable that, if, for any reason, differentiation should start at the distal extremity of the mass, it might work back under the guidance of social pressure toward the base. The distal differentiation would have to get

started in the right direction, or something else than the missing organ would be produced. That cases of heteromorphosis sometimes occur might be interpreted as the result of such failures. But the comparative rareness of heteromorphosis makes me suspect that the beginnings of visible differentiation that frequently appear at the tips of regenerating organs do not occur without any relation to the basal part. The fact that, with few exceptions, such as the failure to regenerate the intermediate segments of the appendages, etc., the whole organ, nothing more nor less, is regenerated, and forms a congruent union with the basal part, is indicative of close interaction of the various parts of developing organ with the body of the organism at all stages of the process.

I am inclined to think that neither centrifugal nor centripetal differentiation, expresses the entire truth of the matter, but that the new part differentiates as a whole, much as organs do in embryonic development, and at all times in intimate functional relations with the old part, differentiation becoming accelerated in one part or another, according to special conditions. If differentiation began at the tip of the rudiment of an organ, and proceeded centrally, the whole might be differentiated before the body was reached, leaving a mass of unused tissue between; or differentiation might reach the body before all the immediate parts were produced. If differentiation proceeded in the reverse direction, similar imperfections might arise. We must look upon a regenerating mass of tissue as one in which incipient developmental tendencies are proceeding in various ways, modifying each other, and gradually working into a condition of physiological equilibrium with the basal part and with the environment before much outward evidence of differentiation makes its appearance. It is probable that the main elements of a regenerating appendage of an arthropod, for instance, are blocked out before any external marks become visible. Even during the early stages of proliferation of the cells of the regenerating appendages, it is not improbable that incipient differentiations are becoming established. And the basal part notwithstanding the fact that the visible differentiation may take place in a centripetal direction, may exercise a guiding influence at all times over the regeneration of the part,

and determine that it forms in harmony with the rest of the organism. Such a conception is entirely congruous with the symbiotic theory, and is, I believe, consistent with the various observed facts of regeneration.

If we explain form regulation as an outcome of functional regulation, we make little progress until we have some interpretation of the latter process, and any theory of form regulation which offers nothing in this direction, makes no more than the first step toward an explanation of the phenomenon. In his criticism of the theory of form regulation which I have outlined, Child has advanced arguments which are, I believe, by no means fatal to it, and he has not brought forward any other explanation of functional equilibration, which both of us regard as the basis of form regulation. Perhaps this may be supplied in further developments of his theory which Child hints are to be made in the future. While functional adaptation may occur independently of any symbiotic relations, especially in the direct adaptation of parts to external conditions, the mutual adaptation of parts which forms so important an element in formative processes are, I believe, for the most part, dependent on symbiotic relations. At present I am unable to see how any general explanation of functional equilibration among the parts of an organism can be reached unless we assume that the parts are, to a considerable degree, interdependent. Perhaps some other interpretation of functional regulation may be advanced which does not make use of this idea. That remains, of course, to be seen. But the theory of the symbiotic relation of the parts of an organism has the merit of enabling us to interpret form regulation and functional regulation as the outcome of ordinary physiological activities, and hence to give, in a measure, a causal explanation of the teleological behavior which is manifested in so striking a degree by formative processes, and which forms the strongest support of some recent vitalistic theories. So far, at least, I hope it is in the line of progress.

## SOME FACTORS IN THE DEVELOPMENT OF THE AMPHIBIAN EAR VESICLE AND FURTHER EXPERI- MENTS ON EQUILIBRATION

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WITH SIX FIGURES

In a previous paper concerning experiments on the developing ear vesicle<sup>1</sup> it was shown that the group of cells forming the primitive epithelial ear cup or ear vesicle of the tadpole is specialized to that degree that although removed to an abnormal environment the cells still continue to differentiate themselves into a structure possessing many of the features of a normal labyrinth. Recently it has been shown by Lewis<sup>2</sup> that even earlier, while still an uninvaginated plate, the ear anlage is already capable of a certain degree of independent differentiation. In the following paper additional evidence will be given of the high degree of developmental independence possessed by the early labyrinth cells. It will be pointed out that individual parts of the vesicle may develop independently of the rest of the vesicle. It will also be shown that the process of differentiation extends to the difference existing between a right and left-sided organ. A left ear vesicle transplanted into the empty pocket left by the removal of the right ear vesicle develops into a labyrinth that is perfect in general form and in its relations to the brain, with the exception that it maintains its left-sided character; the anterior semicircular canal is found on the caudal side toward the vagus group, while the posterior canal lies toward the eye, and likewise the lagena which

<sup>1</sup> Streeter, G. L., '06: Some experiments on the developing ear vesicle of the tadpole with relation to equilibration. *Jour. of Experimental Zool.*, vol. iii.

<sup>2</sup> Lewis, W. H., '07: On the origin and differentiation of the otic vesicle in amphibian embryos. *Anatomical Record*, No. 6, *Amer. Jour. of Anat.*, vol. vii.

normally buds out from the caudal border of the saccule in these cases is found extending forward toward the proötic ganglion.

The ear vesicle, however, is not in all respects independent of the surrounding structures. Some experiments which are reported below, indicate that its position in reference to the brain, ganglion masses and the surface of the body is determined by the environment itself; it may be rotated in any direction, and nevertheless it eventually develops in the normal attitude, with the saccule toward the ventral surface, the semicircular canals toward the dorsal surface, the lateral semicircular canal being toward the lateral surface, and the endolymphatic appendage toward the brain.

The experiments were carried out on larvæ of *Rana sylvatica* and *Rana pipiens*, and the operating stage was the same that was used in previous experiments.<sup>3</sup> The time is just at the close of the non-motile stage, and the epithelial ear consists of an invaginated cup-shaped mass of cells just in the process of being pinched off from the deeper layer of the skin, with the edges turning in to form a closed vesicle. For simplicity the term "ear vesicle" will be used even though the closure is not yet complete; the attempt to distinguish between auditory cup and auditory vesicle does not seem to be justified for the present purposes. The technique of the operations was also the same as that described in the previous paper. Notes were made on the behavior of the animals, and at the end of from four to six weeks the specimens were preserved in a chrome-acetic mixture, cut in serial sections, and stained with hæmatoxylin and congo red. With certain specimens the ear vesicle, adjacent ganglia, and a portion of the central nervous system were reconstructed after the Born wax plate method. Eleven such models were made, and photographs of some of them are reproduced in Figs. 2, 3 and 6. With the aid of these models it was possible to identify relations and detailed features of the labyrinths that otherwise could not have been recognized.

The morphological features of the experiments will be first considered, and the behavior of the animals and its relation to equilibrium will be treated separately in the latter part of the paper.

<sup>3</sup> Streeter '06: *l. c.*, Fig. 3, p. 547.

## DETERMINATION OF POSITION OF THE EAR VESICLE

The conclusion that the attitude of the developed labyrinth, the position of its canals and various chambers, is determined by its environment is based on seventeen experiments in which the ear vesicle was loosened from its normal situation and placed in an abnormal attitude, and the specimen then allowed to continue in its development. At the end of a month examination showed that the labyrinth had become differentiated with varying degrees of completeness, and in each instance had developed in normal relation to the surrounding structures.

*Rotation in Two Directions.* In eight of these experiments the ear vesicle was rotated  $180^{\circ}$  around both its vertical and transverse axes, so that it was turned face inward and upside down; or, in other words, its lateral or invaginated surface was toward the brain and its ventral border was where the dorsal border should be, the maximum displacement. After this procedure the wounds healed within a few hours, and the larvæ were reared up to the fourth or fifth week, when they were killed and cut in serial sections. The labyrinths of five specimens were reconstructed. Before describing them reference should be made to the normal condition of the labyrinth at this age. A reconstruction of a normal one with its adjacent structures is shown in Fig. 1.

From the reconstruction of a normal specimen it can be seen that the three semicircular canals have individual characteristics by which they can be separately identified; such as the Y-shaped union of the anterior and lateral canals, and the overlapping of the caudal end of the lateral canal by the posterior canal, and the junction of the posterior and anterior canals to form the crus commune. The differentiation between utricle and saccule is not yet complete, but the part that is to become saccule is so labeled. From the caudal border of the saccule can be seen a small pocket budding out which constitutes the lagena or primitive cochlea. Directly median to the crus commune is the endolymphatic appendage, consisting of a small duct leading from the main labyrinth chamber up between the labyrinth and brain to a rounded pouch, the saccus endolymphaticus. In their histology, as well as in



their general form, the various parts of the labyrinth exhibit at this time individuality. (See Fig. 4.) The ventro-median portion of the vestibular sac and the ampullar ends of the semicircular canals possess high columnar cells forming the neuro-epithelial maculæ which are supplied with fibers from the acoustic ganglion, lying against the medial wall of the labyrinth. The endolymphatic sac has cuboidal cells, and the lagena has intensely staining columnar cells like those seen in the macular regions. The lagena is further characterized by its sharply rounded outline, and by the fact of its being compactly surrounded by ganglion cells and fibers, and cartilage forming cells. These features are so definite that

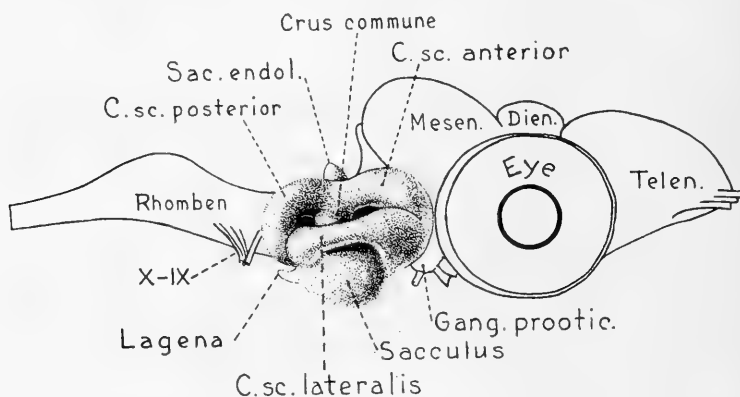


Fig. 1 Reconstruction showing the form and relations of the membranous labyrinth of a normal tadpole (*Rana pipiens*) one month old. The labyrinth, adjacent ganglia and part of the brain were reconstructed after the Born method, and the remainder of the figure was drawn from a dissection of a tadpole of the same age. Enlarged 35 diameters.

the various parts of the labyrinth can be recognized without difficulty, even though they happen to be incomplete, or out of their normal relations.

Now if one examines the models of the operated specimens, photographs of three of which are reproduced in Fig. 2, it is seen that the individuality of the semicircular canals can at once be identified. In model *a*, the canals are practically normal; in model *b*, the anterior canal is small, and the lateral canal consists only of a pouch which has not been pinched off from the main cavity; in model *c*, the posterior canal remains a simple pouch, while the

anterior and lateral canals are normal. In considering the posture of the canals it is to be noted that the surrounding structures have been left out in Fig. 2, to avoid unnecessary duplication; the three models are all represented in the same relative position as that of the labyrinth in Fig. 1, *i. e.*, the cephalic end is on the right, the caudal end is on the left, the ventral surface is below, and the dorsal surface is above. Thus it will be seen that the lateral canals in all three models are in the same plane; likewise the posterior canals all form the dorso-caudal border of the labyrinth, and the anterior canals form the dorso-cephalic border. The fact that the anterior canal is small in model *b*,<sup>4</sup> and the posterior canal is small in model *c*, gives rise to a false impression of a backward

sac. endolymph.

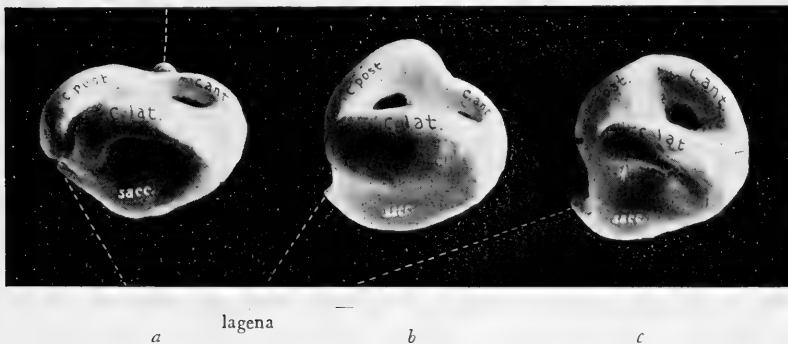


Fig. 2 Reconstructions showing the form and posture developed by three labyrinths one month old which while primitive ear vesicles were rotated from their normal position so as to lie face inward and upside down. The models are placed so that their planes are parallel with those in Fig. 1. Thus they present a lateral view with the cephalic end toward the right, caudal end toward the left, dorsal surface above, and ventral surface below. Enlarged 50 diameters.

and forward tilting of the vesicle. The saccule and lagena have the same position as in Fig. 1, and the lagena points caudally as it should do. The endolymphatic appendage lies on the median side of the crus commune; in models *b* and *c* it is small, but the tip

<sup>4</sup> This may be due to injury received at the time of operation. Such localized defects are frequently seen. They may involve any part of the labyrinth, and they vary greatly in the extent of the labyrinth wall affected. In one case the entire labyrinth was defective, with the exception of the endolymphatic appendage, which was normal in structure and position, and presented a curious appearance, being attached to the small irregular vesicle representing the labyrinth. Such localization of abnormal development is evidence of the high degree of specialization of the cells forming the primitive ear vesicle.

of it can be seen in model *a*. The acoustic nerve and ganglion are attached to the median and ventral surfaces of the labyrinth, and the nerve connection with the brain appears to be normal.

The conditions found in the three specimens pictured in Fig. 2 are typical of what is found in the other five specimens examined. They vary in the completeness of their differentiation, some of them consisting of only a vesicle with perhaps a single canal pouch, but in all cases the acoustic ganglion is present on the ventro medial surface, and the macular areas can be recognized. The lagena is present in seven out of eight cases. The endolymphatic appendage developed in six out of eight cases. As regards posture, the rule is that the more perfectly the labyrinth is developed the more accurately its posture corresponds to the normal relations. But even in the most imperfect specimens when the endolymphatic appendage appears it is on the medial surface, and the tendency to canal formation is always on the dorso-lateral surface, and the saccule and lagena appear on the ventral surface. This condition of course applies only to vesicles that have been implanted in the acoustic region as was done in all the above cases.

*Rotation in One Direction.* In four experiments the ear vesicle was rotated  $180^\circ$  around its vertical axis, *i.e.*, turned face inward. These specimens were then reared as in the preceding instance, and eventually cut in serial sections. A reconstruction model of one of them is reproduced in Fig. 3, and if it is compared with Fig. 1 it will be seen that although the vesicle was started in its development with invaginated side toward the brain yet the completed labyrinth has the normal posture. A section of the same specimen is reproduced in Fig. 4, showing the labyrinth surrounded by developing cartilage. The acoustic ganglion is connected in normal manner with the brain and sends peripheral fibers to the thickened floor of the saccule. The endolymphatic sac is in its normal position, and the narrow duct can be seen connecting it with the main chamber of the labyrinth directly median to the crus commune. The series through this specimen show that histologically it is practically perfect. Of the other three specimens one was almost equally perfect, another showed some abnormalities in the formation of the canals and the lagena, and the

fourth was quite imperfect, consisting of only a large vesicle with a thickened epithelial floor connected by a few nerve cells and fibers with the brain.



Fig. 3 Reconstruction of a tadpole labyrinth one month old, which when a primitive ear vesicle was rotated from the normal position  $180^\circ$  in one direction, so as to lie with invaginated side toward the brain. A section through the same labyrinth is shown in Fig. 4. Enlarged 55 diameters.

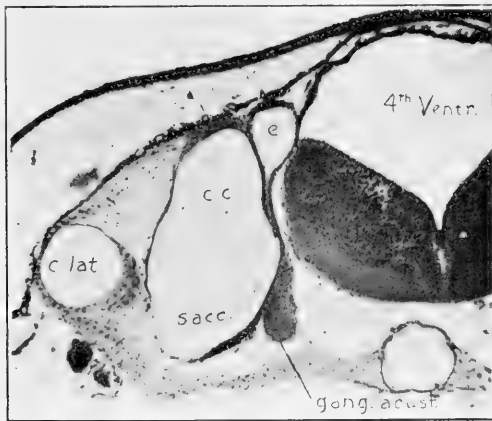


Fig. 4 Section through the membranous labyrinth shown in Fig. 3. It shows that though originally turned face inward it has developed in the normal attitude. *e*, endolymphatic appendage; *c.c.*, crus commune; *sacc.*, sacculus; *c. lat.*, lateral semicircular canal; *gang. acust.*, acoustic ganglion. Enlarged 55 diameters.

*Transplanted Specimens.* The irregularity of form of the six specimens transplanted to the region between the eye and nostril, previously reported,<sup>5</sup> is so great that they give no assistance in solv-

Streeter '06, *l. c.*, p. 557.

ing the question of posture. However, in five cases, which will be presently described, where the ear vesicle was transplanted from the left side to the right side into the place made vacant by the removal of the right ear vesicle, in spite of the fact that these ear vesicles were implanted with haphazard attitude toward the adjacent structures, they nevertheless in each instance developed right-side up, and with the median surface toward the brain, as can be seen in Figs. 5 and 6.

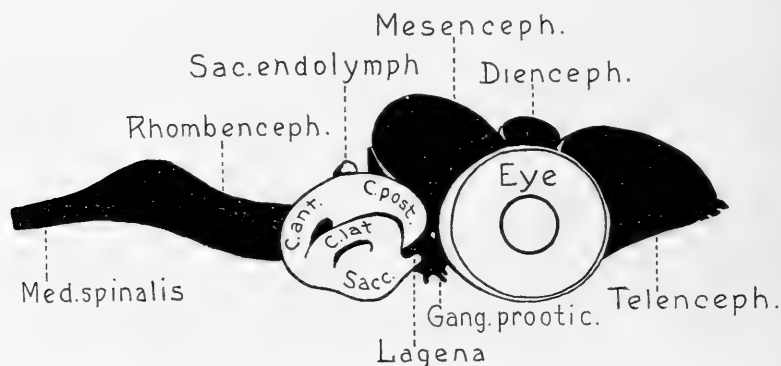


Fig. 5 Reconstruction showing the form and relations developed by a left ear vesicle when transplanted to the right side; it shows that under such circumstances the ear vesicle retains its left-sided characteristics, though it otherwise normally adapts itself to its new situation. A photograph of the same specimen is shown in Fig. 6, c.

#### DETERMINATION OF THE DEXTRAL AND SINISTRAL CHARACTER OF THE EAR VESICLE

The question as to whether the right or left-sidedness of the ear labyrinth is controlled by the environment, or is determined by some intrinsic character of its own constituent cells, is answered in favor of the latter by the fact that if the left primitive ear vesicle, before the time of its complete closure, is transplanted to the opposite side of the embryo it retains its original left-sidedness. In five specimens, at the usual operating stage, the right ear vesicle was removed, and at the same time the left ear vesicle was uncovered and lifted from its natural bed and then placed into the pocket

from which the right vesicle had been taken and allowed to heal. In making the transplantation no effort was made to place the ear vesicles in any particular posture. After keeping the specimens alive for one month they were sectioned and from three of them reconstructions were made of the transplanted ear vesicle together with the adjacent structures. The three labyrinths are shown in Fig. 6, and model *c* is again shown in Fig. 5, with the brain included. It will be seen that in developing they have assumed the normal attitude toward the brain. The endolymph-

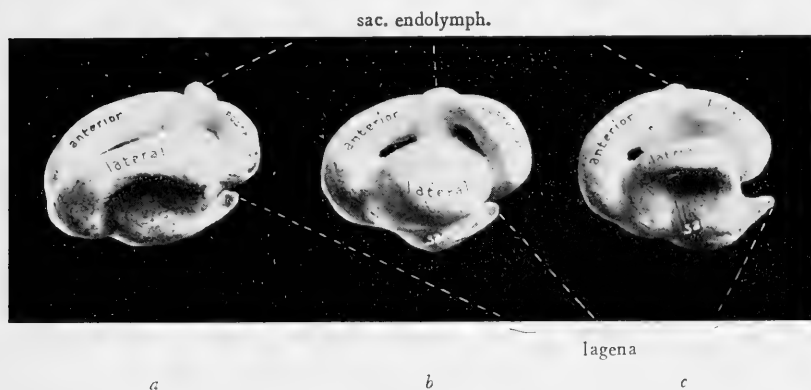


Fig. 6 Reconstructions of three labyrinths which while primitive ear vesicles were transplanted from the left to the right side. They are all represented in the same position as the models in Fig. 2. The model *c* is the same that is shown in Fig. 5. Enlarged 50 diameters.

phatic appendage and the median side of the labyrinth is toward the brain, the semicircular canals are toward the dorso-lateral surface, and the saccule and lagena are toward the ventral surface. But it can at once be recognized that the saccule and lagena point forward toward the eye, and that the anterior and posterior canals are in reversed positions. We thus have a complete mirror image of the right labyrinth, *i. e.*, a left labyrinth. Model *a* possesses three semicircular canals and is almost a normally formed left labyrinth. In model *b* the lateral canal consists of a pouch whose walls did not undergo the customary approximation and central absorption. In model *c* the posterior canal is not pinched off. In each of the models the lagena, saccule, and endolymphatic

appendage are typical, and there is establishment of normal appearing nerve and ganglion connections.

#### EQUILIBRATION

It was found in the experiments performed a year ago that removal of one or both ear vesicles, just after they are pinched off from the skin, produces in the tadpoles definite disturbances in the development of their power of equilibration. It was found that when a tadpole is deprived of but one ear vesicle he is by virtue of the remaining one able to develop practically normal swimming abilities; but when both ear vesicles are removed the results are more serious, and in that case the tadpole never develops any sense of equilibrium and is never able to swim. The loss is not compensated for by any other organ and the animal lies helpless on the bottom of the dish. With one ear vesicle the tadpole swims practically in normal fashion, and with no ear vesicle he cannot swim at all.

The fact that one ear vesicle is sufficient for the maintenance of equilibrium greatly simplifies the study of this mechanism; it means that one side can be immediately eliminated, and the problem is reduced from a bilateral one to a unilateral one. A series of experiments at once suggested themselves, in which the ear vesicle of one side was to be removed, and then various operative procedures undertaken upon the ear vesicle of the opposite side, and the test of its consequent functional ability was to be the very decisive one of whether the animal could swim properly, or whether it could not swim at all.

In the paper referred to there is described the experiment of transplanting the ear vesicle into a subdermal pocket in front of the eye. When this was done the transplanted ear vesicle continued in its development, and in some instances established a nerve-ganglion connection with the forebrain; but such specimens never gave evidence of functional activity. The failure to functionate was not unexpected, inasmuch as the connections established were at an abnormal situation, and furthermore the vesicles though having developed many essential features of the normal labyrinth

were still quite imperfect in the formation of the separate chambers and the semicircular canals. So this year in carrying out the experiments described in the first part of the present paper the behavior of the specimens was eagerly watched, and the endeavor was made to determine the amount of alteration in position and defectiveness in form that is compatible with functional activity, involving the problem of the correlation between function and morphology. The observations made in the different experiments have been arranged and condensed as follows:

*a* Left ear vesicle removed; right ear vesicle loosened from skin and rotated, in six specimens around the vertical axis  $180^\circ$  and in eight specimens around both the vertical and transverse axis  $180^\circ$ . As has already been shown these ear vesicles developed into labyrinths of varying degrees of perfection, some being completely normal in form and having apparently normal ganglion and nerve connection with the brain wall. (See Figs. 2, 3 and 4.) The behavior of all the specimens was uniform, both where the ear vesicle was rotated in one plane and where rotated in two planes; at the end of a week after the operation, when with a normally functioning labyrinth they should be able to swim freely and directly, they instead exhibit only irregular movements or spin around in spirals or circles. Their incoördinate movements continue, and at the end of a month there is no improvement; *i.e.*, they behave exactly like specimens with both ear vesicles removed. Evidently ear vesicles thus treated do not perform their natural function.

*b* Left ear vesicle removed; right ear vesicle fragmented by teasing between the points of two needles, the fragments left in place. Ten specimens were treated in this way, and were kept under observation four weeks, during which time they gave no evidence of any sense of equilibrium.

*c* Right ear vesicle removed; left ear vesicle transplanted to the empty pocket on the right side. Five specimens were operated upon and observed for one month, at the end of which time they were cut in serial sections, and it was found that the ear vesicles had developed into fairly complete labyrinths, but had maintained the characteristics of a left-sided organ. (Figs. 5 and 6.)



Throughout the whole period of observation they had exhibited incoördinate movements, and at the end of that time they were unable to swim. This and the two previous operations indicated that rotation of an ear vesicle, or transplanting it from one side to the other, or fragmenting it was not compatible with the development of its function, in spite of the fact that the ear vesicle proceeded in its development and had become to all appearances almost a perfect labyrinth. In the next experiments less severe treatment was tried.

*d* Left ear vesicle removed; right ear vesicle uncovered and carefully lifted out and then immediately placed back in its original position, the effort being made to do a minimum amount of injury. Of six specimens all exhibited symptoms of the absence of all sense of equilibrium.

In the experiments *a*, *b*, *c* and *d* there was the possibility of injury to both the nerve-ganglion connection and the ear vesicle. In the following experiments the effort was made to restrict the injury to one or the other.

*e* Left ear vesicle removed; right ear vesicle uncovered and a fragment cut from the cephalic portion of its wall, care being used not to otherwise disturb the vesicle. Eight such specimens were kept five weeks, and none of them developed any sense of equilibrium, or were able to swim.

*f* Left ear vesicle removed; right ear vesicle uncovered and a small piece cut from its caudal border, any further disturbance being avoided as in *e*. Eight specimens were operated upon, and after keeping them four weeks none of them could swim properly.

*g* Left ear vesicle removed; longitudinal incision made through skin on right side just dorsal to ear vesicle, and needle passed down between the neural tube and ear vesicle and moved backward and forward so as to sever its nervous connection without otherwise disturbing the ear vesicle or loosening it from the skin. None of the four specimens studied swam properly, though one of them could swim somewhat, but was easily confused by any excitement and then made wild and ill directed movements. It was thought that the ear vesicles in these cases would escape injury; but examination of the specimens when cut in serial sections

showed that they were not perfectly normal. This experiment might be repeated on a larger number of specimens and still greater care used in severing the nerve connection, in which case a perfect labyrinth could doubtless be obtained.

*h* (*Rana catesbiana*) Left ear vesicle transplanted into another specimen, in a subdermal pocket in the region of the protic ganglion between the right eye and ear vesicle, thus the host had three ear vesicles, two being on the right side. Twelve days after the operation three out of four specimens so treated exhibited incoördinate movements. Here we have to consider the crowding out of position of the normal right ear vesicle by the one transplanted near it.

*i* Left ear vesicle removed; fine needle passed through the skin so as to make a small puncture in the right ear vesicle; on withdrawal of the needle the edges of the wound immediately close and there is no loss of cells from underneath or from the skin itself. Of four specimens at the end of one month three were able to swim, and this demonstrated the functional ability of an ear vesicle thus treated.

*j* Left ear vesicle removed; small section of the covering skin removed so as to expose the right ear vesicle, but otherwise it is not disturbed and the nerve ganglion connection is left intact. Five specimens were kept under observation for one month, and four of them behaved throughout like those possessing one untouched normal ear vesicle; except for slight incoördination brought out by excitement they could swim properly.

On bringing together the results of these experiments, it becomes immediately apparent that almost any operative procedure carried out on young larvæ in the region of the ear vesicle seriously interferes with the development of the function of that organ. It is possible to lift a skin flap and expose it, and to make a needle puncture in it without destroying its subsequent usefulness; but any operation involving a loss of part of its wall or disturbing its position and nerve-connection with the brain causes apparently complete loss of function. The functional disturbance is out of all proportion to the histological condition. There may be a labyrinth that to all appearances is perfectly formed and that seems to

have a normal nerve ganglion connection with the brain at the proper place, and yet the specimen may not have given signs of any functional activity on the part of that organ.

Spemann<sup>6</sup> is doubtless mistaken in attributing the disturbance in equilibrium simply to the alteration in the planes of the canals. He reports some experiments in which at an early stage a skin flap was turned back, and the ear vesicle taken out and replaced in various positions; and in such specimens he observed faulty equilibrium, and on sectioning his material the vesicle seemed to lie in an abnormal position, and this he assumes to be the cause of the abnormal movements observed. On the one hand, wax plate reconstructions of misplaced ear vesicles show that in my cases they regain their proper position, and the canals eventually lie in their normal planes; the specimens nevertheless continue to make incoördinate movements. On the other hand, in those experiments where the normal position of the vesicle, as regards the planes of space, was undisturbed the results were equally serious. My own experiments suggest that the difficulty lies not so much with the end organ as with the central connections, and perhaps further experiments in that direction would furnish additional information upon this subject.

#### CONCLUSIONS

The primitive ear vesicle of the tadpole may be loosened from its normal position and rotated in various directions, so that its axes lie in abnormal planes, and notwithstanding such interference it eventually develops into a labyrinth which is right side up and exhibits the normal relations to the brain and the surrounding structures. When transplanted to the opposite side of the body, if placed in the acoustic region, it likewise assumes a normal posture. Judging from these facts, the posture of the labyrinth is controlled by its environment.

The "laterality" of the labyrinth is determined before the closure of the ear vesicle. When the left ear vesicle is transplanted

<sup>6</sup> Spemann, H., '06: Ueber embryonale Transplantation. Verhandl. der Gesell. Deutscher Naturf. u. Aerzte. 78 Vers. Stuttgart.

to the right side it retains its characteristics as a left-sided organ, though it otherwise adapts itself to its new position in a normal manner.

The functional disturbance, in experiments on the ear vesicle, is out of all proportion to the histological appearances; any operation carried out in the acoustic region involving a loss of part of the wall of the ear vesicle, or disturbing its position, or nerve connection with the brain results in faulty equilibrium; absence of function was observed in cases where the labyrinth and its nerve connections seemed to have attained perfect histological development.



# COMPENSATORY MOTIONS AND THE SEMI-CIRCULAR CANALS

BY

BENJ. C. GRUENBERG

WITH TWO FIGURES

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## I REACTIONS OF THE FROG TO MOVEMENTS OF ROTATION

When a frog is slowly turned in a horizontal plane by moving or rotating the vessel in which the frog is at rest the animal turns its head in a direction opposite to that of the rotation. When the rotation has proceeded beyond a certain point, the frog will jerk the head back into alinement with the body, and then again turn it in the opposite direction, and so on, as long as the rotation is continued. The existence of this back-jerk or "nystagmus" is specifically denied by von Cyon ('97, pp. 45, 73) and by Lyon (1899, p. 86), and has been overlooked by other observers. When the frog is restless or active, it will frequently jump or walk in a direction opposite to that of the rotation, bringing the head and body into alinement; then turn the head again and follow this movement with a jump, and so on, while the rotation is continued. But when the frog is fairly quiet, there is always a back-jerk.

When the vessel containing the frog is tilted on a transverse horizontal axis, the animal nods its head up or down, according as the rotation is upward or downward anteriorly. When the base upon which the frog rests is tilted on the longitudinal (horizontal) axis—too slowly to dislodge the animal—the movements

of the head are such as tend to keep the plane of the mouth horizontal; that is, the animal raises the head on the side that is lowered, and vice versa. (At the same time there is a contraction in the limbs on the ascending side and a corresponding flexion on the descending side.) There can, of course, be no question of a nystagmus in these two cases, since the rotation cannot be continued beyond a small arc of a circle without dislodging the animal or causing it to make definite efforts to hold its own, and in neither case are there compensatory responses to rotation. Nor are the responses normal if the animal is fastened to the support.

When a frog is moved about in a circle having a diameter of two to three meters (by walking about with a jar containing the animal), the animal turns its head away from the center if the frog faces the direction of motion, and toward the center if the animal is carried facing in the direction opposite to that of the movement, that is, backward. This movement and the response are virtually the same as in the case of rotation about a vertical axis, in a large circle and at a slow rate.

The movement of the head in the cases referred to is in general in a direction opposite to that of the displacement of the body. Such responses have long been known in the frog<sup>1</sup> as well as in other animals, and are frequently spoken of as "compensatory movements." The implication of this designation, as well as the expressed belief of many physiologists, is that the movements in question are in some way related to the orientation of the animal with regard to gravity, or, what is mechanically equivalent, to acceleration of motion in some direction. The movements have been regarded as reflexes set up by the sensation of the semicircular canals.

## 2 THEORIES OF THE FUNCTION OF THE SEMICIRCULAR CANALS

The oldest theory as to the function of the semicircular canals was that they were concerned in the perception of the direction of sound, and was deduced from their intimate anatomical asso-

<sup>1</sup> Some of these responses seem first to have been described by Goltz ('69, p. 71), and many of them have been shown by Steiner ('85, p. 126) to take place in frogs whose fore- and mid-brain have been removed.

ciation with the other auditory organs and the fact that the three canals of each side lie in planes almost exactly at right angles to one another; that is, in planes corresponding to the three dimensions of space. It can be readily shown that the perception of the direction of sounds is actually accomplished otherwise; and this theory as to the function of these organs has been long abandoned.

In 1828 Flourens (1 and 2) made the observation that, as a result of cutting one of the membranous canals in a pigeon, the bird moved about an axis at a right angle to the plane of the injured canal; that is to say, the movement was in the plane of the divided canal; the sense of hearing, however, was in nowise affected. The disturbed movements were so much like those resulting from injuries to the cerebellum that Flourens concluded that the canals were concerned in the coördination of movements; but he made no attempt to explain the method of their operation.

In 1870 Goltz offered an explanation of the method of the working of the semicircular canals. According to this theory it is the downward pressure of the endolymph on the various parts of the sensitive lining of the membranous canal, according to the position of the head, that gives rise to the corresponding sensations. This theory has been called the "hydrostatic theory."

A few years later, Breuer ('74, '75) and Mach ('75) proposed hydrodynamic theories of the action of the canals. According to Mach the sensations in the canals are aroused by variations in the pressure of the endolymph in the ampullæ, and the variations in pressure result from the streaming of the endolymph, which is caused by movements of the head. According to Breuer it is the movements of the endolymph, resulting from the movements of the head, that arouse the corresponding sensations, through their pressure on or movement of the lining hairs of the canals.

About the same time Crum-Brown ('74, 1, 2 and 3) urged the view that the movement or pressure of the perilymph was as much concerned in the production of the sensation as the disturbance of the endolymph; and he also pointed out that the canals operate in pairs.



In 1878 von Cyon rejected both the static and the dynamic theories of the workings of the semicircular canals. After drawing off the endolymph and replacing it with gelatin, and after the introduction of pieces of laminaria into the canals, thus producing great changes in pressure, there were none of the disturbances of equilibrium that had been observed by Flourens as resulting from divisions of the canals. Without advancing any other explanation of how these peripheral organs *are* excited, von Cyon maintained that the canals assist but indirectly in giving the organism a knowledge of space relations; the sensations in the canals set up reflexes in the eye muscles, and it is from the sensations of the eye muscles and the retinal images that the notion of spatial relations of the head and of the body are obtained.

In 1883 Sewall ('83) from experiments on skates and sharks concluded that the results were not sufficient to warrant the opinion that the semicircular canals are the organs of equilibration

### 3 THEORETICAL OBJECTIONS TO THE SEMICIRCULAR CANAL HYPOTHESIS

Whatever the real manner of operation of the semicircular canals may be, there have appeared certain theoretical objections to Goltz's static theory as well as to the various dynamic theories; these explanations seem to be out of harmony with the observed fact that the responses of the frog's head to rotation are not coördinated with the position of the animal in relation to the axis of rotation. Thus, in Fig. 1, a frog in any one of the four positions on the turntable, will always turn his head to the left if the table is turned to the right (clockwise), and vice versa, as indicated by the dotted outlines and the peripheral arrows. The action of gravity, or acceleration, in relation to the frog, or whatever dynamic principle it may be that does act, seems to work in a different direction in each of the four cases. The special sense of the action in each case is indicated in Fig. 1 by a small arrow. In other words, the animal responds uniformly to what is apparently a variety of stimuli; the stimuli in these cases are the same in kind and in degree, but differ in sense or direction, or incidence in the animal's body;

but the response to every stimulus is the same in degree and in direction.

Lyon ('99, p. 89) has already called attention to this anomalous appearance, and the facts had been observed much earlier (Cyon '97, p. 42; Ewald '90, Fig. 51; Schäfer '87), and had caused great confusion largely because the earlier writers described the responses with reference to the periphery and the axis instead of

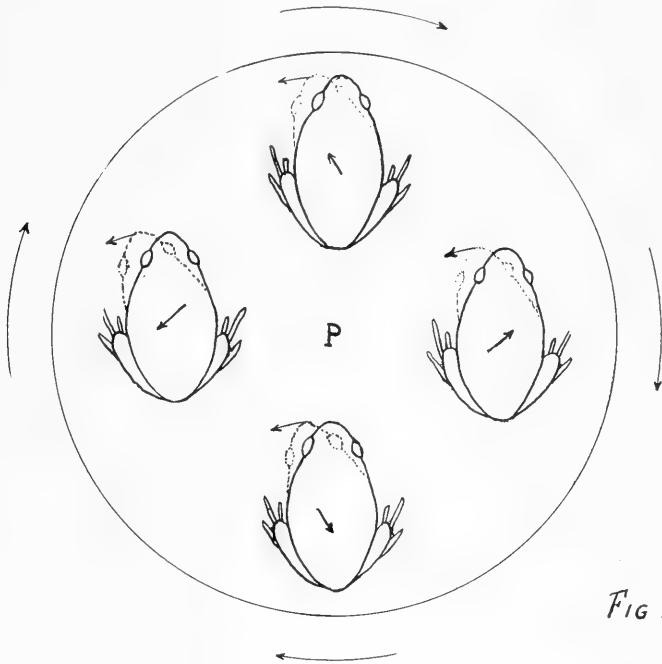


FIG 1.

with relation to the body of the animal. The anomalies and confusions did not lead directly to a rejection of the hypothesis that the semicircular canals were the peripheral organs for perceiving acceleration or spatial relations. Yet these theoretical considerations would seem to make the semicircular canal hypothesis of equilibration untenable without radical modification. The experimental evidence is conflicting and inconclusive.

## 4 EXPERIMENTAL OBJECTIONS TO THE HYPOTHESIS

On removing the semicircular canals entirely, or on cutting the acoustic nerve, Girard ('92) and Schrader ('87, p. 87) report complete loss of compensatory motions; while Tomaszewitz ('77), Breuer ('75, p. 99), Baginsky ('81), Cyon ('88), Kreidl ('92, 2), Ewald ('92), Strehl ('95, p. 216), Bechterew ('96), and others, found all the phenomena in response to rotation continued as in the normal animal; and Steiner ('85, '89) concludes that there is not complete loss of compensatory movements. Breuer ('91), Ewald and Delage-Aubert ('88) observe, however, that the compensatory movements disappear after the operation if care is taken to exclude the use of the eyes also, and therefore they do not abandon the theory; but Cyon ('97) speaks sarcastically of the logic of this argument.

When single canals only were operated upon, Hensen ('79) found the movements disturbed in the plane of the canal in question; whereas Ewald ('90, Experiment 66) cauterized the membranous canals in pigeons without in any way affecting the movements of the animals.

According to Girard and Ewald, the destruction or removal of the labyrinth on one side of the head caused the frog to take on an unsymmetrical attitude, the head and body being inclined toward the operated side; Ewald found that this new attitude was maintained in one case for a year after the operation. According to Loeb ('91, 2) cutting the acoustic nerve brings about a permanent tendency in the shark to turn toward the injured side. On the other hand, Cyon, Steiner ('89), Baginsky ('85) and Bechterew ('96) found on cutting the acoustic nerve that "all the phenomena that served to support the assumption of the sensory function [of the semicircular canals] continued to appear" (Cyon, '97), while Mach found that under these conditions the eye- and head-nystagmus appeared as in normal animals.

Breuer ('89) found that mechanical, thermal and electrical (galvanic) stimulations of separate canals set up head-turnings in the corresponding planes; the movements are in response to the streaming of the lymph, and are in the same direction as the stream-

ing. "Galvanic dizziness \* \* \* is caused by irritation of the vestibular nerve endings, as galvanic phosphorescence is produced by irritation of the retina." Lee ('93, '94, '98) found the responses to stimulation of the canals and ampullæ such as to lead to the conclusion that the canals are directly concerned in equilibration. Mach's theory that compensating movements are set up by variations in the pressure of the endolymph seems to be disproved by the experiments of Cyon ('88, pp. 294-297), Spamer ('80) and Ewald ('90, Experiment 42), who secured normal reactions after producing permanent changes in the pressure of the endolymph by removing the liquid entirely, by replacing it with gelatine or with amalgam, and by inserting into the canals dry bits of laminaria, which swelled up on absorbing moisture, and so increased the pressure.

On the one hand Ayers ('92) draws from his morphological studies the conclusion that the canals are specially modified lateral-canal organs, that have no relation whatever to equilibration. On the other hand Schaeffer ('94) tells us that whirling produces no effect upon tadpoles until after the semicircular canals become developed. But Streeter ('06) has succeeded in separating the action of the canals from that of the rest of the ear vesicle and concludes that while the ear vesicles are essential to the development of the power of equilibrium in tadpoles, the canals are not.

Schäfer ('87) succeeded in demonstrating to his own satisfaction that the responses to rotation are due solely to the inertia of the loosely jointed head; he made a wooden model that behaved on the turntable just like a frog or a pigeon, with a few exceptions to be noticed later.

Other minor experiments have been reported by various investigators, with equally definite but conflicting and inconclusive results.

## 5 ANALYSIS AND NEW EXPERIMENTS

This then appears to be the situation:

1 From the structure of the semicircular canals it was inferred that they were somehow related to the perception of *space* or *direction*.

2 The manner in which the semicircular canals operate to bring about perception of space has been variously explained as resulting from (*a*) static inertia of the endolymph; (*b*) variation in pressure of the endolymph; and (*c*) movements or acceleration of the endolymph, or of the perilymph, or of both; brought about by movements of the head.

3 Theoretical considerations seem to show that this function cannot be ascribed to the canals for the reason that identical reactions are produced under conditions in which the sense of the acceleration may be opposite. (As explained in connection with Fig. 1.)

4 But operations to remove or destroy the semicircular canals, or to sever the connections of the VIII nerve, show (*a*) in some cases that the movements of the animals are seriously affected, and (*b*) in other cases that the animals continue to respond to rotation as do the normal animals.

5 Mechanical, thermal and electrical stimulations of the single canals show (*a*) in some cases decided disturbances of movement related to the planes of the respective canals, and (*b*) in other cases the absence of related responses.

On examining again the movements represented in Fig. 1, it will be seen that a given rotation will produce for the frog a displacement of the retinal image or "view," and always in the same direction without regard to the position of the animal on the turntable. Whereas the actions of centrifugal force and of acceleration depend upon the position of the animal with relation to the axis of rotation, the sense of displacement of the field of vision does not so depend, and it may therefore be supposed that the uniform turnings of the head are in response to the changing view; the frog seems to be trying to keep the same view before him.

To test the responsiveness of the frog to the apparent displacement of his surroundings, a "revolving environment" was arranged, consisting of a cylinder of stout paper about 60 cm. in diameter and about 35 cm. high, attached to a wooden hoop which was suspended so that it could be readily rotated in either direction. A portion of the cylinder consisted of light colored material bearing black vertical stripes about 5 mm. wide and from 2 to 5

cm. apart. Another portion of the cylinder was of black paper in which had been cut numerous holes of various shapes and sizes. When in use the cylinder was always part striped and part fenestrated, or part open and part one or the other of the described surfaces. (The proportions were varied, but the character of the surface did not seem to make a constant difference.)

Frogs placed in the middle of this "circus" arrangement could be made to turn their heads and to give the nystagmus or back-jerk by revolving the cylinder, the same as when the animals themselves were rotated on the turntable. The response was not, however, equally marked in all cases, nor was it in any case as quick as in the actual rotation of the animal. When the revolving of the cylinder was very rapid or very slow, there was no response at all; but when the optimum rate was found, the responses were well marked and continuous. These experiments with the moving environment would indicate that the visual impressions do, or may, play an important rôle in setting up compensatory movements; and in the case of the animal rotated on the turntable one might conclude that it is the displacement of the retinal image that is the constant, and therefore the determinant factor. But such a conclusion would be false, and for the following reasons:

If a frog is placed on the turntable, in every possible position with relation to the pivot, and the table is turned to the right, (that is, clockwise) the frog's head will always turn to the (animal's) left, and the animal will seek, humanly speaking, to keep the same view before his eyes. But if now the vessel containing the animal is completely surrounded by some opaque material, the frog will respond in precisely the same way. If the frog is taken into a room almost dark—one barely light enough to permit the observer to discern the outlines of the animal against a white background—the animal will respond in the same way. If the animal is placed upon the turntable together with the source of illumination, and completely cut off from the sight of external objects, rotation will result in the same reactions. If, finally, the animal's eyes are covered with a mixture of vaseline and lamp-black (which will entirely exclude vision without in the least irritating the frog) the responses to rotation are still the same.

It may accordingly be safe to conclude that while the turnings of the head on rotation may be responses to visual impressions, they may also be quite independent of visual impressions. One is therefore driven back to a reëxamination of the semicircular canal theory, or to search for some other means of perceiving movement or acceleration.

It had already been found that there is not complete loss of the compensatory movements on cutting the acoustic nerves,<sup>2</sup> or on destroying the semicircular canals. This is comprehensible, since the eyes are *also* capable of leading to similar results. If a frog that has been operated upon is rotated with the eyes covered, or surrounded by some opaque medium that rotates with him, there is no response. This excluding of visual impressions is not, as Cyon supposed, eliminating *the* determining factor, since the normal frog under the same conditions will continue to react, though Cyon failed to observe this. The results referred to in this paragraph I have verified experimentally.

The following is the record of one frog whose semicircular canals had been destroyed by piercing into the capsule from the dorsal side. The animal was etherized; there was no bleeding.

1 Immediately after the operation (the animal recovered consciousness and began to move about sluggishly within two or three minutes after the operation):

*a* Animal lies on back quietly over one-half minute at a time without making efforts to right itself.

*b* Rights itself only with great difficulty and after making many awkward movements.

*c* Limbs not correlated in crawling about; does not hop.

*d* On turntable, no response.

*e* In swimming, rolls from side to side.

2 After thirty minutes:

*a* Lies on back for short intervals, but not quietly.

*b* Rights itself with difficulty, but more quickly than at first.

*c* Moves about awkwardly, but better than at first; can hop, but in jumping frequently lands on back.

<sup>2</sup>Vide *supra*.

*d* On turntable responds as normal, but more slowly.

*e* Responds as normal to "revolving environment."

3 After one week:

*a* Lies on back indefinitely, quietly.

*b* Rights itself more easily than before, but still awkwardly.

*c* Walks about unsteadily; leaps awkwardly, falling on side.

*d* On turntable, responds normally.

Another frog, with both sets of semicircular canals destroyed by boring into capsule from the dorsal side, showed after three weeks a marked lack of coördination of movements, though not as great as at first; this was evident in swimming as well as in walking, and in both swimming and in jumping the animal frequently turned over on its back; it righted itself rather quickly, but movements still showed awkwardness. On turntable, responses were as in the normal animal.

As has been pointed out above, the summation of mechanical disturbances or accelerations on the rotation of an animal upon the turntable in a given direction seems to depend upon the position of the animal with reference to the axis of rotation, whereas the sense of the response in relation to the animal's own axis is constant. Thus, the rotation being to the right (clockwise), the factor of wind, or resistance of the air, acts upon the *right* side of the animal if the animal faces the periphery, but on the *left* side of body if the animal faces the pivot; but in any case the response is to the *left*. The same apparent contradiction is observed if we consider the direction of the centrifugal force of the rotation; the direction of the centrifugal pressure of the viscera or other loose parts, of the strains on the skeletal articulations, and of the friction of the body on the supporting surface, is toward the periphery, however the animal may be placed; but the response to a given rotation is constant with reference to the axis of the animal. The same apparent contradiction is found when the attention is directed to the inertia of the viscera or of the contents of the semicircular canals. In addition to these contradictions is the further fact pointed out by Schäfer in 1887 and by others, and referred to above (§4), that the inertia of the head, because of its loose articulation to the trunk, is sufficient to account for the



"responses" to rotation even in a wooden frog; and these responses agree in sense with those described for the live frog. That is to say, with a given rotation of the turntable the "turning" of the head is constant, without regard to the position of the body in relation to the axis of rotation. These considerations in detail have led many physiologists to abandon the theory that the canals are the organs for the perception of movement or acceleration, since they so obviously arouse the same response to opposite sets of stimuli, and since the responses can be obtained from wooden animals as well as from Nature's own. *But there is one element in the mechanical theory that seems to have been overlooked as a constant source of rotation stimuli.*

The inertia of a loosely jointed head as an explanation of the phenomena may be left entirely out of account because in the first place it cannot account for the back-jerk, in the second place the inertia is overbalanced by the centrifugal force when a certain rate of rotation is reached, whereas the responses do not disappear at this point, and in the third place the responses can be inhibited by stimuli that do not seem to affect the freedom of the head articulation.

Steiner ('85) has described the reactions of the frog in response to rotation essentially as given here, and analyzed the movements in terms of tangential force; but he concludes from the persistence of the reactions after the division of the eighth nerve, that the canals are not concerned in the matter at all. It is to be noticed that when the animal is rotated on a turntable, the posterior end of the body is constantly changing its position with reference to the anterior end, as is the right side with reference to the left, etc. This movement is constant in direction, and parallel (in direction) to the rotation. That this is a real motion and quite distinct from the motion of translation or rotation, is known to every physicist and to some laymen; but the physicist has a name for it.

Prof. Albert P. Wills, of the Department of Physics of Columbia University, has kindly helped me to get the matter clear by assuring me that this kind of motion is well recognized in mechanics, and by giving me the technical name for it. It is known as the "spin." This *spin* it is that remains constant in direction on

a given rotation, unaffected by the position of the animal on the turntable; when the rotation is clockwise, the spin is clockwise, and vice versa; when the spin is to the right, the head of the frog turns to the left, and vice versa. It is the "spin," therefore, that determines the compensatory movement.

To test the validity of this interpretation, it is necessary to eliminate this factor from the rotation of the animal. For this purpose an eccentric was arranged on the turntable in such a

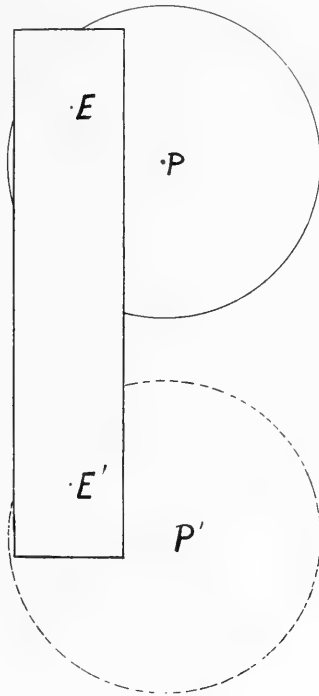


FIG 2

manner that the animal could be moved in a circle with its long axis always parallel to its first position (Fig. 2).

When the frog is rotated on this eccentric turntable, the response takes the form of a pendulous movement of the head; during one portion of the revolution the head is turned to the right, and then the head is turned in the opposite direction. This back and forth swinging of the head continues as long as the rotation

is continued, and there is no nystagmus whatever. The head turns to the left in that part of the rotation which carries the animal's body to its right, and vice versa, whether the rotation be clockwise or the reverse, and without regard to the size of the circle described; that is, without regard to the proximity of the animal to one of the pivots. If the animal is placed in an opaque vessel and the rotation on the eccentric set up, there is no response whatever.

To show the effects of the various factors that have been considered as having a possible relation to the "compensatory movements," the following comparative table may be helpful. In the experiments whose results are given under I the animal was

	I		II	
	A	B	C	D
	Facing Periphery	Facing Pivot	Facing Periphery	Facing Pivot
1 Air pressure	←	→	←	→
2 Inertia (of viscera, lymph, etc.)	←	→	←	→
3 Centrifugal action	↑	↓	↑	↓
4 Friction of support	→	←	→	←
5 Displacement of retinal image	←	←	←	→
6 "Spin"	⤿	⤿	○	○
Response	←	←	←	→

rotated to the right (clockwise) on the ordinary turntable; in those represented under II the eccentric arrangement was used and the results given are for a portion of the revolution only, since a continuation of the rotation beyond  $180^\circ$  is virtually equivalent to a reversal of the motion. (It is of course understood that the rotations in the reverse direction gave corresponding results but in the opposite sense.) The arrows indicate the directions in which the respective factors are supposed to act.

On comparing the arrows in the four columns it will be seen that whereas all in II seem to be related to the direction of the head turning, none in I are so except 5 and 6 (retinal and spin impressions).

In experiments on the turntable (I) the factor 5 could be eliminated in a variety of ways: By surrounding the vessel containing the frog with some opaque material, or placing it in a tall opaque cylinder; by covering the eyes with the opaque non-irritant mixture already referred to, or with a pad of absorbent cotton mixed with vaseline and lampblack; by placing the source of light on the turntable with the animal. In all cases the turning of the head in response to rotation was the same as in the usual rotation as to direction; but frequently it was less in degree. In other words, while the displacement of the retinal image can and does set up the compensatory response, the eye is not the sole sense organ through which such movements can be initiated.

This leaves the spin as the only other factor to be further considered. According to the results indicated in columns A and B of the table, the spin is the only factor (of those considered) in addition to vision that can constantly set up the head turning. In the experiments on the eccentric (columns C and D) where the spin is already eliminated, the further elimination of sight results in a total loss of the response.

The slightest amount of spin is sufficient to set up a perceptible amount of head turning; considerable displacement of the retinal image is required to bring about the same amount of response. It is possible to move the frog in a right line without the animal giving any response whatever; but if the movement is not smooth, that is, if there is vibration, or very slight turning in a horizontal plane, the head responds at once. That the response to the spin is quicker and greater in amount is also certain; the two factors may be caused to operate in opposite directions in the following manner:

A dish holding the frog on a horizontal plane and facing the observer, is swung about slowly by the observer at arm's length. The head will be seen to turn in the *same* direction as the movement of translation; that is, in a direction opposite to what we

should expect on the anthropomorphic view of the animal "seeking to keep the same vision in sight." But the turning of the head is opposite to the direction of the spin that the observer unconsciously imparts to the dish in moving his arm outstretched, which is thus in the radius of a horizontal rotation.

That the perception of spin or rotation is located in the organs of the inner ear seems likely from the fact that the response is eliminated when the semicircular canals are destroyed or removed, or when the acoustic nerve is cut. That the sensation concerned involves a factor of rotation or turning is indicated by the fact that rectilinear acceleration does not yield the same constant response.

It may, therefore, be concluded that the compensatory movements of the frog's head set up by rotation arise in response to two distinct sets of stimuli, visual and dynamic; that the response to the visual stimulus is relatively feebler and slower than that to the dynamic stimulus; that the organ for the perception of the dynamic factor is probably located in the internal ear; and that the dynamic perception involves a rotation or turning element in the stimulus, as distinguished from an acceleration or movement in a single direction.

## 6 SUMMARY

1 There is apparent contradiction between the various responses of the frog to rotation on the turntable and any theory of mechanical stimulation of peripheral organ as the origin of the responses.

2 There is considerable contradiction among various experiments that have been made in connection with the relation of the semicircular canals and compensatory movements.

3 A reëxamination of the compensatory movements and of the conditions under which they arise shows the presence of a mechanical factor, the "spin," the significance of which in this connection seems not to have been considered before.

4 From an examination of the results obtained by earlier observers, a repetition of some of their experiments, and new experiments made in the course of the study, the following conclusions are drawn:

*a* The compensatory movements of the frog's head set up by rotation arise in response to two distinct sets of stimuli, visual and dynamic.

*b* The response to the visual stimulus is relatively feebler and slower than that to the dynamic stimulus.

*c* The organ for the perception of the dynamic factor is probably located in the internal ear.

*d* The dynamic perception involves a rotation or turning element in the stimulus, as distinguished from an acceleration or movement in a simple direction.

I wish to express my sincere thanks to Prof. T. H. Morgan for the helpful suggestions and encouragement that have made this study possible; and to Prof. A. P. Wills for his assistance in elucidating to me the mechanics of rotation.

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# A STUDY OF THE SPERMATOGENESIS OF TWENTY-TWO SPECIES OF THE MEMBRACIDÆ, JASSIDÆ, CERCOPIDÆ AND FULGORIDÆ, WITH ESPECIAL REFERENCE TO THE BEHAVIOR OF THE ODD CHROMOSOME<sup>1</sup>

BY

ALICE M. BORING

WITH NINE PLATES

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<sup>1</sup>A dissertation presented to the Faculty of Bryn Mawr College for the degree of Doctor of Philosophy.

## INTRODUCTION

The purpose of this investigation is to extend, to some families of the Hemiptera Homoptera, the studies of McClung, Stevens, Wilson and others on the relation of the accessory or odd chromosome to sex determination. Except for the aphids, which have been extensively worked out by Stevens ('05a, '06a), *Cicada tibi-cens* (Wilcox '95) and *Aphrophora quadrangularis* (Stevens '06b) are the only species of this group whose spermatogenesis has been previously described. This study covers eight species of the Membracidae, six of the Jassidae, four of the Cercopidae and four of the Fulgoridae.

My work was begun at the suggestion of Dr. N. M. Stevens at Woods Hole in the summer of 1905, continued under Prof. E. G. Conklin, at the University of Pennsylvania, in the year 1905-06, and completed under Dr. Stevens, at Bryn Mawr College, in the year 1906-07. To both Dr. Stevens and Professor Conklin I wish to express my appreciation of their valuable suggestions and constant help and inspiration. I wish also to thank Dr. Herbert Osborn of Columbus, Ohio; Mr. E. P. Van Duzee, of Buffalo; Mr. H. C. Barber, of New York City, and Dr. H. Skinner, of Philadelphia, for the identification of material.

## HISTORICAL REVIEW

Most of the work on the spermatogenesis of the tracheate arthropods has been done since 1890. Such studies as those of Bütschli ('71), La Valette St. George ('85), Platner ('86), Verson ('89), and Sabatier ('85) were concerned only with the formation of the spermatozoa, the arrangement of the cells of the testis into cysts, and the general mechanics of karyokinesis. The work of van Beneden ('84), Boveri ('87) and O. Hertwig ('90) on *Ascaris*, and Mark ('81) on *Limax*, turned the interest in the study of the sex cells to the chromosomes, while Weismann's daring hypothesis ('87) as to equational and reducing divisions added to the interest. By 1890, practically all investigations on spermatogenesis centered around the chromosomes in the spermatocyte divisions, and in

that year we find the first statement that one chromosome behaves differently from the others (Henking '90). Unfortunately there is the greatest confusion in the results for the next decade; but since Montgomery's suggestion ('01a) that synapsis means the conjugation of homologous maternal and paternal chromosomes, and its confirmation by Sutton's work on *Brachystola* ('00, '02, '03), there has been greater accord. As a consequence of this, certain fundamental theories are coming to rest on a firm foundation. The chromosomes are shown to keep their individuality from one cell generation to another. The real reduction in number is proved to be brought about by the joining of each paternal to a corresponding maternal chromosome in synapsis. It is found to make no difference whether the reducing or equational division comes first, but the distinction between these two divisions is constant, the one being the separating of the individual spermatogonial chromosomes, the other a simple splitting of these univalent chromosomes. In addition to this, recent work indicates that there is usually present throughout the Tracheata an odd chromosome in the spermatogonia, which behaves differently from the other chromosomes throughout its history. Still later work seems to establish the fact that this chromosome has no paternal mate, does not join any other chromosome in synapsis, divides in only one spermatocyte division, and enters only half of the spermatozoa. In some forms, a small chromosome is present as the paternal mate of this odd chromosome, but dimorphism of the spermatozoa results in either case.

The following review takes up the different observations on the Tracheata since 1890, and attempts to show how each helps to establish, or differs from, the above mentioned theories.

### *Arachnida*

Wallace ('05) finds an even number of spermatogonial chromosomes, 40, two of these being larger than the others and different in behavior. They are condensed in the spermatogonial rest stage, and take an eccentric position in the equatorial plate. They remain separate from each other in the spermatocyte growth

period and do not divide in either spermatocyte division, as the other 19 chromosomes do, thus appearing in only one quarter of the spermatozoa. Wallace concludes that all the spermatozoa degenerate except those with the two odd chromosomes.

Montgomery in *Lycosa* ('05) finds an even number of chromosomes in the spermatogonia. Two of these he calls heterochromosomes, although the only characteristic that justifies this name is that they remain condensed in the growth period. They conjugate like the other chromosomes and divide in both divisions, all of the spermatozoa receiving one-fourth of the heterochromosome tetrad.

The results of neither of these investigators agree with the more recent work on the odd chromosome in spiders and other forms. If, as Wallace states, no spermatozoa develop except those containing the two odd chromosomes and the nineteen ordinary chromosomes, the eggs must all contain only 19 chromosomes, as the spermatogonial number is 40. Suppose each egg to have 19 chromosomes; fertilization by a spermatozoön with  $19 + 2$  chromosomes would give all the offspring  $38 + 2$  ( $19 + 2$  in the reduced number), whether male or female; but according to Wallace's contention, the egg can have only 19; therefore it is impossible that all the spermatozoa, except those with the two odd chromosomes, degenerate. According to Montgomery, the heterochromosome in the spermatocyte is bivalent and divides in both divisions. Berry's work ('06) brings the odd chromosome in the spider into line with the odd chromosomes in other forms; it is a single chromosome in the spermatogonia, and divides in only the second division of the spermatocytes, resulting in dimorphism of the spermatozoa.

### *Myriapoda*

Blackman ('05a, '05b) finds in *Scolopendra heros* and *S. subspinipes* an uneven number of spermatogonial chromosomes. Synapsis takes place in the late anaphase of the last spermatogonial division, all of the chromosomes uniting in pairs except the odd one. The odd chromosome divides only in the second spermatocyte division. The peculiarity here is that the other chromosomes

seem to undergo their reducing division when the odd chromosome is dividing equationally, but this is only a further mark of the individuality of the chromosomes, and does not furnish any evidence against Montgomery's theory of synapsis. Medes ('05) finds a similar condition in *Scutigera* forceps.

### *Orthoptera*

Neither vom Rath ('91, '92) nor Wilcox ('95) noticed an odd chromosome in *Gryllotalpa* or *Caloptenus*, although both mention a nucleolus in the spermatocyte growth period which may be the same structure. They both insist that there are two reducing divisions; that is, two divisions that separate whole chromosomes from each other. This is probably due to a confusion in the use of the word chromosome. If we use the terminology suggested by McClung ('00), univalent chromosome in the spermatogonium, bivalent chromosome in the spermatocyte, and chromatid for each unit of the tetrad, the discrepancies in the work of vom Rath and Wilcox are cleared up. Vom Rath finds 12 spermatogonial chromosomes. In the growth period, the spireme splits into six rods, each of which forms a tetrad, or divides into four "chromosomes," as he expresses it. As he calls each chromatid a chromosome, he considers that he has two divisions which separate chromosomes from chromosomes; and therefore must be reducing; while in terms of the original spermatogonial chromosomes, one division is reducing and one equational. Wilcox falls into the same difficulty; he finds 12 spermatogonial chromosomes, and then the spireme divides into 24 "chromosomes," which form 6 tetrads. He had, in reality, 24 chromatids, and only one reducing division.

McClung ('00, '02a) has described the odd chromosome in the *Acrididæ* and *Locustidæ*. He worked on a number of forms and obtained uniform results. In the *Orthoptera*, this chromosome can be traced back into the spermatogonial rest stages. It divides only in the first spermatocyte division, giving dimorphism of the spermatozoa. In 1901, McClung suggested the theory which has since that time received substantial corroboration, that the dimor-



phism of the spermatozoa corresponds to the dimorphism of sex. McClung considers that the longitudinal division always precedes the reducing division, and thinks that this is important on account of the failure of the second polar body to be extruded in parthenogenetic eggs; but the work in the other groups of insects shows that the reducing division probably comes first as often as the equational.

Sutton's careful work ('00, '02) on *Brachystola magna* offers convincing evidence for the individuality of the chromosomes. Each pair of spermatogonial chromosomes becomes enclosed in a separate compartment of the nucleus, while the odd chromosome is in a vesicle shut completely off from the others. He suggests the application of Montgomery's theory of the union of maternal and paternal chromosomes in synapsis to Mendelian inheritance.

The observations of de Sinéty ('01) on the odd chromosome in one of the Acrididæ and in several Phasmidæ are entirely in accord with those of McClung; this chromosome divides in only one spermatocyte division, producing dimorphic spermatozoa. In one of the phasms, he finds a chromosome complex similar to that described later by McClung ('05) for *Hesperotettix*, where the odd chromosome attaches itself to one end of a tetrad, forming a hexad which divides along the transverse axis of the tetrad, thus sending the odd chromosome and two chromatids of the tetrad to one cell, and two to the other. Unfortunately de Sinéty interprets both of the spermatocyte divisions as longitudinal, but on this point he is in the minority among the workers on Orthoptera.

Baumgartner ('04), in *Gryllus domesticus*, finds the odd chromosome in a separate vesicle as Sutton did for *Brachystola*, but he finds it dividing in the second division instead of the first. Stevens ('05a) in *Stenopelmatus* and *Blatella germanica*, and Otte ('06), in *Locusta viridissima*, find that the odd chromosome divides in the second division instead of the first. Evidently there is no fixed rule as to where the odd chromosome shall divide.

Voinov ('03), Montgomery ('05) and Zweiger ('06) all hold a different view as to the valence of the orthopteran odd chromosome; but as each has studied only one species of the order, while

the work of McClung, de Sinéty, Sutton, Baumgartner and Stevens covers numerous species in several families, we have a right to question the views of these other three observers. All three hold that the heterochromosome which they describe is formed from two spermatogonial chromosomes and divides in both spermatocyte divisions.

Moore and Robinson ('05) claim that the odd chromosome in *Periplaneta americana* is only a plasmosome which dissolves before each division and is reconstructed after it.

#### *Odonata*

The paper of McGill ('04) on *Anax junius* seems to show the same confusion which Wilson has discovered in Paulmier's work on *Anasa tristis*. McGill finds an even number of chromosomes in the spermatogonia, two of them small. These she identifies with the chromatin nucleolus of the rest stage and the odd chromosome, which divides in the first division and not in the second. If it could be shown that there are only 27 chromosomes in the spermatogonial plate, and that the odd chromosome is one of the larger ones, this form would fall into line with other work.

#### *Lepidoptera*

The early investigators in this field, Platner ('86) and Verson ('94) paid no attention to the chromosomes. I have not been able to read Toyama's papers, but the references to them by McClung indicate that the work is not very satisfactory. Stevens ('06b) gives a few figures for two species. There are two condensed bodies throughout the growth period, which fuse in prophase like the *m*-chromosomes in *Alydus* (Wilson, '05c), and this body divides in both divisions like the equal "idiochromosomes" of *Nezara*.

#### *Coleoptera*

The only work on the *Coleoptera* which deals with the heterochromosomes is that of Stevens ('05b and '06b) and of Nowlin ('06). Some of the beetles have an odd chromosome and others have an unequal pair in which the large member of the pair is the

maternal homologue of the odd chromosome, and the small member is the paternal mate which is lacking with the odd chromosome. In the Coleoptera, the reducing division comes first, the equational second. In this order of insects there is substantial proof of McClung's sex determination theory, as the oögonial equatorial plates have been shown to have the large chromosome, while the spermatogonial plates have the small one, and there is the same difference between the somatic plates of the males and females. The theoretical bearing of these facts will be discussed later.

### *Hemiptera*

The chromosomes in this group are so large and few in number that they have attracted many workers, but in spite of this fact, there have been greater discrepancies than in almost any other group. Henking ('90) in working on *Pyrrhocoris apterus*, was the first to notice that in one spermatocyte division, one chromosome does not divide, thus causing a dimorphism of spermatozoa. He counted 24 chromosomes in the spermatogonia, and thought that this odd chromosome had the same valence as the others. He observed a large darkly-staining nucleolus in the growth period, although he did not associate a chromatic nature with it, or connect it with the odd chromosome of the spermatocyte mitoses. He formulated no theory to account for the dimorphism of the spermatozoa.

Wilcox ('95) records that there are 12 spermatogonial chromosomes in *Cicada tibicens*, and 24 spheroidal bodies in the spermatocytes, instead of a reduced number, results similar to those on *Caloptenus femur-rubrum*.

In *Anasa tristis*, Paulmier ('99) describes two small spermatogonial chromosomes, which form first the chromatin nucleolus in the growth period, then a tetrad which divides in the first spermatocyte division, and not in the second. Because this chromosome is small and appears in only part of the spermatozoa, he regards it as degenerating chromatin. Wilson ('05c), working over the same field, finds that Paulmier has confused two bodies, inasmuch as the two small chromosomes form a tetrad and divide in

both divisions, while the odd chromosome, which divides only in the first division, is the chromatin nucleolus of the rest stage and one of the large chromosomes of the spermatogonia. He maintains that Paulmier made a mistake also in the spermatogonial number, which is always odd. Foot and Strobell ('07), by the use of smear preparations and photo-micrographs, have attempted to show that Wilson is in error in his observations on the spermatogenesis of *Anasa*. They find that the odd chromosome acts essentially like any other chromosome, is made up of two spermatogonial chromosomes and divides in both spermatocyte divisions, its only peculiarities being that it does not appear as a tetrad in prophase and occasionally divides later than the other chromosomes in metaphase. They attempt to show that the chromatin nucleolus of the rest stage is not a chromosome, but dissolves before metaphase like a plasmosome. Wilson ('07) has carefully gone over his preparations and still thinks that his former conclusions are correct. There is need of more work with smear preparations to test their reliability.

Gross ('04), in his work on *Syromastes*, apparently confuses the *m*-chromosomes with the odd chromosome much as Paulmier did. In *Pyrrhocoris apterus* ('06) he finds the odd chromosome bivalent but dividing in only one spermatocyte division.

Montgomery ('01a) calls the odd chromosomes of the Hemiptera "chromatin nucleoli" and considers that they may vary in number and valence. He explains them as chromosomes on the way to disappearance during progressive evolution. His results show many discrepancies which have since been explained by Wilson ('05b and '05c).

Wilson groups the Heteroptera into three classes, those with an unequal pair of heterochromosomes, those with an odd chromosome and *m*-chromosomes, those with an equal pair of heterochromosomes. In the first class, the chromosome number in the second spermatocyte is one less than in the first spermatocyte. This is due to the fact that the conjugation of the unequal pair does not take place until after the first spermatocyte division. This is the most direct evidence yet found for Montgomery's synapsis hypothesis, for the small chromosome can be proved to be paternal,

and the large one, maternal. In the second class, the odd chromosome is homologous with the large maternal element in the unequal pair. The *m*-chromosomes are a pair, whose synapsis is delayed until just before the first spermatocyte division. The third class includes forms where there is neither an unequal pair, nor an odd chromosome, and therefore no visible dimorphism of the spermatozoa, but the fact that the equal heterochromosomes do not conjugate until after the first spermatocyte division, relates this class to the first class, and suggests that there may be a masked dimorphism, the equal heterochromosomes representing different characters, possibly, as truly as the unequal heterochromosomes where there is a visible dimorphism. Wilson cites a great deal of evidence for the individuality of the chromosomes, finding the same size relations between pairs of spermatogonial chromosomes as there are between single chromosomes in the spermatocytes. He elaborates McClung's sex determination theory, brings forward much evidence for the dimorphism of the spermatozoa, and shows that there is a corresponding dimorphism in the somatic equatorial plates of the male and female of several species of the Hemiptera heteroptera.

#### MATERIAL AND METHODS

My material was collected at Woods Hole in the summer of 1905, at Cold Spring Harbor in the summer of 1906, and at Bryn Mawr in the fall of 1906. The insects were caught in the usual sweep net, and the testes dissected out as soon as possible. Each testis consists of a group of several follicles, each attached by a separate duct to the vas deferens. The testes from the larvæ just ready for metamorphosis, and from the adults soon after metamorphosis, in most cases give all stages from the spermatogonia to the mature spermatozoa.

Before putting up material of any species, Schneider's aceto-carmine proved to be a quick and efficient reagent for determining whether the testes contained all the important stages. This fixes and stains the material at the same time. The testis is put on a slide in a drop of the stain, and the cells separated by pressing down the coverglass. The preparation is made air-tight

with vaseline, and in a few minutes, the chromatin is stained a deep carmine. The entire spermatogenesis might be worked out in such preparations, the only disadvantage being that the achromatic structures are not well fixed, and the preparations are not permanent. Camera drawings made from the aceto-carmine material, compared with those from sections of material fixed in the usual reagents, show the chromosomes in the former much larger in size. (Compare Fig. 198 with Fig. 205, and 201 with 207.) This difference is largely due to shrinkage in the usual fixing fluids and alcohols. The relative sizes and positions of the structures are the same in both kinds of preparations.

If the material showed the right stages, it was put up in various fixing fluids: Gilson's mercurio-nitric, Flemming's strong chromo-aceto-osmic, Hermann's platino-aceto-osmic, and Carnoy's acetic alcohol with sublimate. The dissecting was usually done in the fixing fluid, but the small quantity of material that was dissected in physiological salt solution and immediately transferred to the fixing fluid, showed just as good fixation, as is shown by the clear outlines of all the cell structures. A few cases of poor fixation were apparently due to the long time the insects were kept in captivity, as was sometimes necessary when the material was collected several miles from the laboratory, and immediate dissection was impossible. Gilson's mercurio-nitric was the fixative used most frequently, because it gives excellent fixation of the chromatin and is a very convenient fluid to use, but nearly all material was also put in one or both of the osmic mixtures, as these give better fixation of the achromatic structures. The Gilson was used for two to six hours, the Flemming and Hermann for twelve to twenty-four hours, followed by the same length of time in running water. The Carnoy was used but little. It does not fix so well as the Gilson. Its real value is for material where an aqueous fixative cannot be used.

After fixation, the material was run through the alcohols, cleared in xylol, and embedded in paraffine with a melting point of  $52^{\circ}$  C. Most of the sections were cut  $5\ \mu$  thick, a few  $3\frac{1}{2}\ \mu$  and  $6\frac{2}{3}\ \mu$ .

Many stains were tried. The three giving most satisfactory

results were Heidenhain's iron hæmatoxylin, either without a counterstain, or with a slight tinge of orange G, thionin without a counterstain, and Auerbach's combination of acid fuchsin and methyl green. With iron hæmatoxylin, the long method gave the best results. Preparations in this stain furnish the best outlines for camera drawings, but for work in spermatogenesis, there is the disadvantage that it often stains plasmosomes and chromosomes alike. Thionin has proved a valuable stain for distinguishing between chromatic material and plasmosomes. With this material the best results are gained by leaving the slides in the stain from one to five minutes, rinsing off with water, and differentiating under the microscope with 95 per cent alcohol. The basichromatin holds the stain as a navy blue or dark purple, depending upon the material; while the plasmosome and oxychromatin either take a very pale blue, or hold no color at all. The Auerbach stain also gives differentiation between basi and oxychromatin, the odd chromosome standing out bright green in the rest stage against the pink spireme or scattered oxychromatin.

#### OBSERVATIONS

##### *Membracidæ*

In the *Membracidæ*, the testes are situated ventrally, near the anterior end of the abdomen. They are white in color, and each follicle is round. Such ripe spermatozoa as are present are found near the duct and the spermatogonia are situated on the opposite side. The rest of the follicle is filled with the intermediate stages, grouped into cysts containing cells in about the same stage. The succession of these stages is rather difficult to follow in the *Membracidæ*, because the follicles are spherical and no one longitudinal section gives all of the stages. The only way to trace the development is to find cysts with most of the cells in one stage and a few in transition to the next stage. In this way, the links between the stages can be filled in. In the eight species from which my material was obtained, the general course of development is very similar, with only here and there a striking difference. I shall there-

fore describe in detail one species, *Entilia sinuata*, and then mention the chief points of interest in the other species.

### *Entilia sinuata*

This form was found in September, at Woods Hole, on the leaves of the Golden Glow, and later near Philadelphia, on the wild sunflower.

The resting spermatogonia stain very lightly, as there are only a few basichromatin granules in the midst of much scattered oxychromatin (Fig. 1). When the cell is preparing for division, a heavy, rather darkly-staining spireme is formed with the chromatin aggregated at regular intervals along the linin (Fig. 2). A longitudinal split appears in this spireme, a slight indication of which can be seen in Fig. 2. The chromatin next becomes condensed and segmented, but these segments still retain their linin connections. The longitudinal split in each segment is also very conspicuous at this stage (Fig. 3). Condensation of the segments continues, there being first an elimination of the longitudinal split (Fig. 4), and then a shortening of the segments until they are about twice as long as broad, the form which they have as they enter the equatorial plate of the spindle (Fig. 5). They appear in the plate with their longitudinal axis at right angles to the longitudinal axis of the spindle and with the linin connections still intact. This division, therefore, is a longitudinal division, separating each chromosome into two parts along the line of the original longitudinal split, which appeared in prophase. A lateral view of the spindle in metakinesis also shows convincingly that this division is longitudinal (Fig. 6). The number of chromosomes in the spermatogonial division is 21 but it is impossible to pick out the odd chromosome. The chromosomes become so closely massed together in anaphase (Fig. 7) that one cannot tell whether the linin connections still remain intact, or the conjugation of chromosome pairs takes place here. By the time the cell division is completed, the new nuclear membrane has been formed, possibly as Conklin ('02) has suggested, by the joining together of the linin sheaths of the chromosomes after these have absorbed liquid from the cytoplasm (Fig. 8). A linin connection joining the chromo-



somes end to end is visible soon after they have lost their smooth contours (Fig. 9).

The last spermatogonial telophase is followed by a dense, darkly-staining contraction stage, which looks like a tightly wound spireme. Here the outlines of the chromosomes and their connections are entirely obliterated. The contracted mass occupies only a part of the nucleus, leaving a large clear space at one side (Fig. 10). This space appears in preparations where the fixation of other parts seems to be perfect, so it can hardly be looked upon as an artefact, as McClung ('00) at first claimed. I have used Wilson's ('05b) expression, "contraction stage" as simpler than McClung's "synizesis," for the most condensed period of "synapsis" as Moore used the term. The chromatin now goes through a series of changes comparable to those of *Anasa tristis* (Wilson '05c): (1) an early postsynapsis, with a fine spireme, much twisted on itself, still staining deeply, but filling the nucleus much more completely than in the contraction stage (Fig. 11); (2) a late postsynapsis, with the spireme filling the cell completely, less twisted, and staining unevenly (Fig. 12); (3) an early growth stage, with the spireme thicker, the basichromatin aggregated at regular intervals along the linin (Fig. 13); (4) a rest stage, where the spireme scarcely stains at all, and in the midst of the pale nucleus (in iron hæmatoxylin) there is one lens-shaped black body (Fig. 14), which, following Stevens, I shall call the odd chromosome. It is the "accessory of McClung, the "chromatin nucleolus" or heterochromosome" of Montgomery, the "chromosome spéciale" of de Sinéty, or the "heterotropic chromosome" of Wilson. From the action of similar bodies in related species, I am convinced that it must be present here in the postsynapsis and early growth stages, but the spireme stains so deeply and twists on itself so much that it hides the odd chromosome. In the succeeding stage, where the spireme becomes longitudinally split, the odd chromosome lengthens out and loses the smoothness of its outline, although not the intensity of its staining reaction (Fig. 15). The spireme next divides into ten segments, each retaining its longitudinal split (Fig. 16). Counting the odd chromosome, which remains closely applied to the nuclear membrane, there are now 11 chromatic

elements present in the nucleus. Just before the contraction stage, the spermatogonial chromosomes were joined end to end by linin connections, and out of the contraction stage there came a continuous spireme, which has passed through various stages and finally segmented. If the chromosomes conjugate end to end in the late anaphase (Fig. 8), as Fig. 9 might suggest, the longitudinal axis of the primary spermatocyte segments, or chromosomes, represents the longitudinal axis of the spermatogonial chromosomes. The presence of a massed anaphase and of the contraction stage makes it impossible to prove that this is the case here. It has, however, been proved for other forms (Sutton) and the agreement of all other steps in the process points to a possible similarity in this respect also. The 10 segments next become tetrads by the formation of transverse arms which always remain a little shorter than the longitudinal arms, and thus make it always possible to distinguish between the longitudinal and transverse axes (Figs. 17 to 19). While the tetrads and dumb-bells are forming, the odd chromosome rounds up again and becomes a lens-shaped body, still applied to the nuclear membrane (Fig. 20). It is in the dumb-bell form that the chromosomes usually enter the spindle (Fig. 24), but occasionally they are still in the form of cross-shaped tetrads (Fig. 22). This shows conclusively that the longitudinal axis of the dumb-bell is the same as the longitudinal axis of the tetrad, and that the first spermatocyte mitosis is a transverse division. That it is probably a reducing division can be shown by tracing back the development, and working out the corresponding axes: the division between the halves of the dumb-bell (Fig. 24) corresponds to a division along the lateral arms of the tetrad (Fig. 17), and that to a transverse section of the spireme segment (Fig. 16) and that to the separation of one spermatogonial chromosome from another, if we assume that each spireme segment equals two spermatogonial chromosomes joined end to end. This may be further evidence against McClung's ('00) contention that the reducing division is always the second. In the equatorial plate of the first spermatocytes the odd chromosome stands a little apart from the other 10 chromosomes, and is smaller in diameter (Fig. 21). It does not divide in the first spermatocyte division, but lags

behind the others in going toward the spindle pole (Figs. 25 and 27). The chromosomes mass together in the anaphase, so that as soon as the odd chromosome joins the others, it is no longer possible to distinguish it (Fig. 28).

The spindle fibers stand out very clearly, especially in the material fixed in Flemming or Hermann, and it is noticeable that the odd univalent chromosome is joined to only one pole by its mantle fibers, while the bivalent chromosomes are attached to both.

During the telophase the granules of a "Zwischenkörper" can be seen on some (Fig. 25) or all (Fig. 26) the spindle fibers. These show only in iron hæmatoxylin preparations which have not been extracted very thoroughly. In such preparations the centrosomes of the first spermatocyte division can also be seen (Fig. 23). They divide during the anaphase of the first division (Figs. 25 and 27) in readiness for the second division which succeeds the first without any reconstruction of the nucleus.

The chromosomes rearrange themselves (Fig. 29) into a plane at right angles to the plane of the first division, and soon form a regular equatorial plate. Half of the second spermatocytes contain 10 chromosomes (Fig. 31) and the other half 11 (Fig. 30), that is, 10 plus the odd chromosome. In the cells containing 11 chromosomes, the odd one does not differ enough in size to make it any longer distinguishable. In this division, all the chromosomes in all of the cells divide. The reasons for this conclusion are: (1) the lateral views of the metaphase (Fig. 32) never show one undivided chromosome among the other dividing ones, (2) all the chromosomes are attached by mantle fibers to both spindle poles, and (3) in the anaphase, there is never a lagging chromosome near one pole without a mate at the other pole (Fig. 33). That this division of chromosomes is at right angles to the first, that is, longitudinal and equational, is certainly conditioned by the formation of the spindle which is derived directly from that of the first division. The same fibers between the chromosomes and centrosomes remain intact, and as the centrosome divides, the chromosomes are pulled into an equatorial plate at right angles to the equatorial plate of the first spermatocyte division. This second division therefore corresponds to the preliminary longitudinal splitting of

the spireme in the growth period. One spermatocyte division is reducing and the other equational. In the anaphase, the chromosomes again become massed together (Fig. 34) and the nucleus is reconstructed by the formation of a nuclear membrane (Fig. 35). The "Zwischenkörper" is again noticeable in this telophase.

In the young spermatid (Fig. 36), the chromatin is still massed together and stains deeply. The spindle material remains as the "Nebenkern," as first described by v. La Valette St. George ('86) for insect spermatids. The chromatin soon scatters through the nucleus in definite clumps and it is evident that half of the spermatids contain a smooth round darkly-staining body (Fig. 37), while the other half do not (Fig. 38). Through several succeeding stages, this same fact is noticeable; *i. e.*, when the chromatin becomes more diffuse (Figs. 39 and 40), when it forms a pale network and the axial filament has grown out (Figs. 41 and 42), and even when the chromatin has begun to condense to form the head of the spermatozoön (Figs. 43 and 44). The method of determining whether this body is in only half the cells or in all is as follows: cysts of spermatids in various places were picked out and the number of cells with and without this body were counted in each cyst. In studying sections, it must be remembered that parts of some cells are in another section, so even if this body ( $x$ ) were actually present in all the cells, it would not appear in all in any one section of a cyst. On the same principle, if it were actually in only half the cells, it would appear in less than half in any one section. In Entilia, this body appears in a few less than half of the spermatids. It always takes the chromatin stains, deep blue with thionin, and green with the Auerbach. As it resembles the odd chromosome of the first spermatocyte rest stages in staining reaction and contour, and as it appears in not more than one-half of the spermatids, a condition which the odd chromosome necessarily fulfills from the fact of its not dividing in the first spermatocyte division, we seem to be justified in concluding that the body  $x$  of the spermatids is a derivative of the odd chromosome of the spermatocyte. There is nothing unusual about the formation of the spermatozoön. The "Nebenkern" forms the sheath of the axial filament (Fig. 41), the acrosome differentiates from the cytoplasm at the apex of the

head, the head forms by condensation of the chromatin (Figs. 44 to 47), passing through one rather diffuse stage (Fig. 46).

### *Vanduzea arcuata*

*Vanduzea arcuata* was found in abundance on the locust trees near Cold Spring Harbor in June. The spermatogonial plates show 17 chromosomes, varying in size (Fig. 48). It is not possible to arrange them all in pairs, but at least two large pairs are well marked ( $a_1$  and  $a_2$ ,  $b_1$  and  $b_2$ ). In the growth stage, the odd chromosome appears as a long, darkly-staining body, without a smooth contour. It is at first bent upon itself in different forms (Fig. 49), and later lies at full length along the nuclear membrane (Fig. 50), resembling the same stage in *Entilia sinuata* (Fig. 15). In the equatorial plate of the first spermatocyte division, there are 9 chromosomes, two of which are larger than the others (Fig. 51,  $a$  and  $b$ ), corresponding to the four large ones in the spermatogonial plate;  $a$  is slightly larger than  $b$  just as  $a_1$  and  $a_2$  were slightly larger than  $b_1$  and  $b_2$ . This point certainly counts as evidence that each spermatocyte chromosome represents not an indefinite segment of the spireme, but two individual spermatogonial chromosomes. The odd chromosome can be recognized by its eccentric position. Fig. 52 shows all the chromosomes but  $x$  in metakinesis, and in Fig. 53  $x$  is passing to one pole undivided. Figs. 54 and 55 show variations in the position of  $x$  in anaphase; it does not always lag behind, but may even precede the other chromosomes to the pole. The second spermatocyte equatorial plates, containing 9 and 8 chromosomes, respectively, are shown in Figs. 56 and 57. Each has one large chromosome  $a$ , one not quite so large  $b$ , and six small ones of about the same size. Fig. 56 has a ninth chromosome of intermediate size which must be the odd chromosome, as  $x$  in the first spermatocyte plate has a corresponding intermediate size (Fig. 51). All the chromosomes divide in this division, including the odd one, as is shown in all of the lateral views of the metaphase (Fig. 58) and of the anaphase (Fig. 59). Half of the spermatids contain the odd chromosome, and half do not (Figs. 60 and 61).

*Ceresa taurina*.

Three species of *Ceresa* were found near Cold Spring Harbor on the morning-glory vines and tall weeds, during the last three weeks of July. Unfortunately the chromosomes of the spermatogonial plates in all three forms are too close together to make it possible to count them. They all have the same reduced number of chromosomes and a peculiar deposition of chromatin on the nuclear membrane in the growth period. As this phenomenon is most pronounced in *Ceresa taurina*, I shall give the details for this form. In the contraction stage, the chromatin is massed at one side of the nucleus in a number of darkly-staining loops with their bases united in a dense flat chromatic plate, which stains more deeply than the loops (Fig. 62). As the loops spread through the nucleus, they stain less, making the contrast with the black plate more intense (Fig. 63). In the rest stage (Figs. 64 to 67), the reticulum does not take basic stains at all; the chromatin plate appears in various forms, sometimes continuous and sometimes broken up into two, three, or four parts. By the time a split spireme is formed, it has been almost entirely dissolved (Fig. 68), and in the prophases, no trace of it is left (Fig. 69). When these masses dissolve, the odd chromosome becomes visible as a round, smooth body (Figs. 67 and 68), which probably was concealed in the midst of the chromatic plate as far back as the contraction stage, but its presence was obscured by the similarity of its staining reaction to that of the other chromatin. As to the meaning of this deposition of chromatin on the nuclear membrane, it seems possible that it is basichromatin thrown out from the chromosome loops in the contraction stage, and that it takes no part in the further formation of the chromosomes, since it disappears before the next division. The only case at all similar which I can find in the literature is that of *Gryllus campestris* described by Voinov ('04). He claims that all the chromatin is gathered into the "corps nucleinien double," leaving the non-stainable achromatic substance spread through the nucleus, and that when the spireme forms, the chromatin is added to it again from this structure. He neglects the distinction between oxy and basichromatin, and thinks that when all

the stainable chromatin is aggregated into one body, there is no chromatin left elsewhere. The situation is much clearer if looked at from Conklin's point of view ('02): although the nucleus in the rest stage does not take basic stains, it still contains chromatin in the form of oxychromatin; this has the power of changing into basichromatin to form the chromosomes for division. The basichromatin masses of the rest stage, with the exception of the odd chromosome, which here again shows its individuality by a difference in behavior, are apparently rejected substances, which disappear without playing any further rôle in karyokinesis.

In the prophase, the odd chromosome lies close to the nuclear membrane as in the forms previously studied, and in the metaphase it has a somewhat eccentric position (Fig. 70). The chromosomes here are so nearly of the same size that it is impossible to trace any individuals from cell to cell; but the odd chromosome, by virtue of its position and its univalence, can be followed until the second spermatocytes are formed. Figs. 71 to 73 show its varying behavior in metaphase; it may either follow or precede the other chromosomes to the pole. This fact is shown also by the two anaphase figures, 74 and 75. The second spermatocyte equatorial plates show the two numbers of chromosomes 11 and 10 (Figs. 76 and 77), but the odd chromosome can no longer be distinguished from the others, either in metaphase (Fig. 78) or anaphase (Fig. 79). In all the spermatids (Fig. 80), there appears one large body (*n*) taking the basic stains, probably analogous to the body in the beetle spermatids called a chromatin nucleolus by Stevens ('06b). It is impossible to decide whether the odd chromosome in half the spermatids keeps its individuality as was observed in *Entilia* and *Vanduzea*, for all the chromatin stains deeply and in some stages is broken up into many separate masses (Fig. 80).

#### *Ceresa bubalus*

The only external difference between this species and the foregoing one is its greater size and the different angle of the prothoracic protuberances. The only difference in the spermatogenesis as can be seen by Figs. 81 to 92, is that the mass of rejected chromatin is not so conspicuous. In the bouquet stage (Fig. 81), the

plate is not nearly so large as in the same stage of *Ceresa taurina* (Fig. 63). Fig. 82 represents one of the most extreme cases of the growth stage.

*Ceresa diceros*

The shape and size of this species is about the same as in *Ceresa bubalus*, but the coloring is different, being brown and white, instead of uniform green. The spermatogenesis is practically the same, as Figs. 93 to 101 show, but a preparation from the testis of one could be distinguished from a preparation of the other, because the cells, chromosomes, and spindles of *C. diceros* are always smaller than those in *C. bubalus*.

*Atymna castanea*

This species was found on the chestnut trees exclusively, and was very abundant at the end of June and beginning of July. No spermatogonial plates in which the number of chromosomes could be counted were found. The odd chromosome appears in the rest stage as a large round body with a smooth contour and an affinity for basic stains (Fig. 102). In lateral view of the metaphase of the first spermatocyte division, it is apparent that it does not divide (Figs. 104 and 105), and in the anaphase it has the position usually characteristic of this order, between the plates of chromosomes, but nearer one pole than the other (Fig. 106). The number of chromosomes in the first spermatocyte is again 11 (Fig. 103), two of them constantly larger than the others (*a* and *b*). These two large chromosomes appear in all the second spermatocyte plates, whether they have 11 or 10 chromosomes (Figs. 107 and 108). All the spermatids contain a chromatin nucleolus (Fig. 111), as in the genus *Ceresa*. There being apparently no other basic-staining body in any of the spermatids, the odd chromosome in half of them must take part in the formation of the general reticulum like the other chromosomes.

*Campylenchia curvata*

*Campylenchia curvata* was found in sweepings from various weeds throughout July. The material showed all desirable stages.



Many spermatogonial plates were found, some of which it was possible to count. It seems that there must be one short period in the arrangement of the chromosomes into the plate, when they are spread further apart than at any other time. Judging from the behavior of the chromosomes of the first spermatocyte in coming into the equatorial plate, this more open stage must occur when the chromosomes are first drawn into a flat plate from their scattered position in prophase. Later as metakinesis begins and the mantle fibers pull from the two poles, the chromosomes are drawn closer together and the diameter of the plate becomes smaller. Fig. 112 shows a very clear spermatogonial plate, with 19 chromosomes. It is possible here to group the chromosomes into 9 pairs with one left over; only the two most distinct pairs are lettered,  $a_1$  and  $a_2$ , long and slender,  $b_1$  and  $b_2$ , a little shorter and thicker. The two chromosomes formed by the fusion of these pairs are designated by  $a$  and  $b$  in Fig. 114, the equatorial plate of the first spermatocyte, and in Figs. 117 and 118, the equatorial plates of the second spermatocytes. The number of chromosomes in the equatorial plates are what would be expected after finding 19 in the spermatogonia; 10 in the first spermatocytes, and 10 and 9, respectively, in the second. In the rest stages (Fig. 113),  $x$  appears as usual, but there are also present two other smaller bodies with the same staining reaction,  $m_1$  and  $m_2$ . I have called them  $m$ -chromosomes, as they have all the characteristics of Wilson's  $m$ -chromosomes in the rest stage of the Hemiptera Heteroptera ('05c); they are of equal size and they take the basic stains like the odd chromosome. As unfortunately they are not enough smaller than some of the other chromosomes to be readily distinguished in the spermatogonial plate, or to be traced through the prophase of the first spermatocyte to the spindle, it is impossible to see whether they really represent one pair whose fusion has been delayed. The odd chromosome appears as usual in metaphase (Fig. 115) and anaphase (Fig. 116) of the first spermatocyte division, and as usual is not distinguishable in the metaphase (Fig. 119) or anaphase (Fig. 120) of the second division. In the spermatids, a basic-staining body appears in half the nuclei (Figs. 121 and 122), and so must here (as in *Entilia* and *Vanduzzea*) represent the odd

chromosome, rather than the chromatin nucleolus of the other Membracidæ studied.

### *Enchenopa binotata*

*Enchenopa binotata* was found throughout July at Cold Spring Harbor on the locust and wild cherry trees, on blackberry bushes and sometimes in general sweepings of weeds. Its spermatogenesis has been the most puzzling of any form studied and the following account is given tentatively, with the intention of going over the work as soon as more material can be obtained. The first facts to be noticed are that all the chromosomes appear as dumb-bells in the metakinesis of the first spermatocyte (Fig. 128), there is no lagging chromosome in the anaphase (Fig. 130), and all the second spermatocytes have 10 chromosomes (Fig. 131), the same number as the first spermatocytes. In iron hæmatoxylin preparations extracted to the same degree as in other material, no darkly-staining body appears in the rest stage, but in those extracted for a shorter time, a long twisted body appears against the pale spireme (Fig. 124). This can occasionally be traced into a stage where the spireme has segmented (Fig. 125), but never any further, as it does not assume a compact rounded shape until the other chromosomes become condensed. The question arises as to whether this body in the growth stage represents two spermatogonial chromosomes and consequently divides in both spermatocyte divisions as all bivalent chromosomes do; or whether it is univalent, analogous to most odd chromosomes in insects, but divides in the first spermatocyte division and not in the second, thus differing from all the other Hemiptera Homoptera studied and resembling most of the Heteroptera. There were a few spermatogonial plates in such a stage that it was possible to count the chromosomes, but these did not have the chromosomes as clearly spread apart as in most the other species studied. In five plates, 19 chromosomes were counted (Fig. 123) and in two, 20. One of those with 20 may, however, be deceptive; two of the chromosomes are much smaller than any in the other plates, the plate is at the surface of the section, and as  $x$  in Fig. 123 is V-shaped, it is possible that the bend of the V was cut off and the two small chro-

mosomes may really be but one. Other evidence for the univalence of one chromosome is its occasional appearance in early metaphase of the first spermatocytes when it has not yet assumed the dumb-bell shape (Fig. 129), and a few second spermatocyte metaphases where it apparently does not divide (Fig. 133). If it does not divide in the second spermatocyte division, the second spermatocyte spindle should always appear as it does in Fig. 133 rather than as in Fig. 132, unless the odd chromosome is usually in the center surrounded by the other chromosomes. That this probably is true is indicated by several cases like Fig. 135, the two anaphase groups of one second spermatocyte spindle, *a* having 9 chromosomes and *b* 10. There is a space in *a* corresponding to the chromosome marked *x* in *b*. This evidence is anything but satisfactory, but the possibility of such an exception to the general rule that the odd chromosome divides in the first spermatocyte division, is too interesting a fact to leave unmentioned. Here again one large chromosome in the first spermatocyte (Fig. 126) is represented by two in the spermatogonia (Fig. 123, *a*<sub>1</sub> and *a*<sub>2</sub>), and by one in the second spermatocyte (Fig. 131, *a*). Fig. 127 shows an occasional first spermatocyte with 11 chromosomes, implying a delay in the fusion of one pair. Here we find the chromatin nucleolus in all the spermatids (Fig. 136).

### *Jassidæ*

The testes of the Jassidæ are pale yellow in color, and therefore very easy to dissect out. The follicles are about three times as long as broad; this makes it easier to trace the development from stage to stage than in the Membracidæ. My material includes six species, four of them caught at Cold Spring Harbor in July, and the other two, *Agallia sanguinolenta* and *Phlepsius irrotatus*, at Bryn Mawr in October.

#### *Chlorotetrix unicolor* and *C. vividus*

This material was fixed and preserved as belonging to one species, but study of the sections showed two different reduced numbers of chromosomes, 11 and 9. This led to a careful com-

parison of my specimens with those in the collection at the Academy of Natural Sciences, Philadelphia. There proved to be two species, *C. unicolor* and *C. vividus*, in which the only marked difference is the width of head and thorax. Some of my specimens are slightly narrower than others, so I have probably mixed the two species, and cannot state whether the 9 chromosomes belong to *C. unicolor* or to *C. vividus*.

The resting spermatogonium has a reticulum of oxychromatin and linin and a plasmosome, which stains black in iron hæmatoxylin, but shows its achromatic nature in thionin (Fig. 137). There were no good spermatogonial plates in the material with the smaller number of chromosomes, but a lateral view of the spindle is shown in Fig. 138, and the anaphase in Fig. 139. The chromatin then passes into a contraction stage which is very dense, but contains several clear vacuoles (Fig. 140). This has a very different appearance from the contraction stage of the Membracidæ. A spireme stage follows where the chromatin again fills the nucleus and still stains deeply (Fig. 141). The odd chromosome is first visible in the rest stage (Fig. 142) where the chromatin stains least and is most scattered. It is closely applied to the nuclear membrane as was usually the case among the Membracidæ. The spireme splits longitudinally (Fig. 143), and then becomes segmented (Fig. 144). In all stages the odd chromosome can be distinguished by its small size. In the prophase of the first spermatocyte division, it can be recognized by its rounded contour; in the equatorial plate, by its eccentric position (Fig. 145); in the lateral view of the metaphase (Fig. 146), by its undivided condition; and in anaphase, by its lagging behind at one pole of the spindle (Fig. 147). In the equatorial plates of the second spermatocytes with 9 chromosomes, it can still be recognized by its small size (Fig. 149). As it divides in the second spermatocyte division, there is no indication of it in a lateral view of the metaphase (Fig. 150), or anaphase (Fig. 151). Two of the 9 chromosomes are larger than the others (*a* and *b* in Fig. 145), and they keep their individuality in the second spermatocyte (*a* and *b* in Figs. 148 and 149). In all the spermatids, there is one condensed body, which resembles the body called a chromatin nucleolus in

five species of the Membracidae. In the early spermatid, this is the only condensed body distinguishable (Fig. 152), but later when the chromatin becomes more diffuse, it appears that half the spermatids have another smaller condensed body (Figs. 153 and 154), which is lacking in the other half. This must be the odd chromosome, observed in the same stages of three species of Membracidae. In a still later stage, when the reticulum is arranged around a series of clear vacuoles, this difference is still to be observed; all the cells have the one large body, but only half have the small chromosome (Figs. 155 and 156). After this, both bodies disappear, the chromatin reticulum becomes slightly more condensed at first (Fig. 157), the nucleus then elongates but keeps the vacuoles (Fig. 158), and finally condenses into the head of the spermatozoön (Fig. 159). The acrosome is differentiated from cytoplasm at the apex of the head.

Fig. 160 is the spermatogonial plate of the species with the larger number of chromosomes. It contains 21 chromosomes, four larger than the others, not differing conspicuously in size among themselves ( $a_1$ ,  $a_2$ ,  $b_1$ ,  $b_2$ ). The first spermatocyte equatorial plate has 11 chromosomes, and they show the same size relation as those of the other species, two large ones and one small odd chromosome in an eccentric position (Fig. 161). This plate simply has two more chromosomes of intermediate size than the other. The second spermatocyte plates again show the two large chromosomes (Figs. 162 and 163), the total numbers being 11 and 10, instead of 9 and 8.

#### *Dicrocephala coccinea*

A few scattered individuals were found in July in general sweepings, but in August an abundance of material was obtained from the blackberry vines. The spermatogonial plates show 23 chromosomes, two larger than the others ( $a_1$  and  $a_2$  in Fig. 164). In the postsynapsis stage, the odd chromosome is not surrounded by the spireme, as has been the case in the forms described above, but it stands out distinctly by itself in the clear part of the nucleus (Fig. 165). In the rest stage, it is still of the same size and in the same position, although the nucleus grows much larger and the

chromatin becomes scattered and diffuse (Fig. 166). The first spermatocyte shows the odd chromosome as a medium-sized body, eccentric in the plate of 12 chromosomes (Fig. 167), and not dividing in metakinesis (Fig. 168). In anaphase, it lags behind the others (Fig. 169). The two large chromosomes of the spermatogonia have fused into a single large one in the first spermatocyte (*a* in Fig. 167), and this keeps its individuality in the second spermatocytes (*a* in Figs. 170 and 171). Half the second spermatocytes have 12 chromosomes, and half 11. The spermatids all have the chromatin nucleolus, and half of them the odd chromosome (Figs. 174 and 175), as in *Chlorotetrix*.

#### *Diedrocephala mollipes*

This species resembles *Diedrocephala coccinea* in shape, but not in color, being bright green instead of red and green striped. Its spermatogenesis is also similar (Figs. 176 to 185), but the cells and chromosomes are smaller (cf. Fig. 177 and 167). They both have the same number of chromosomes, 12, but *Diedrocephala mollipes* has no one chromosome markedly larger than the others. The spermatids have both a chromatin nucleolus and an odd chromosome.

#### *Phlepsius irrotatus*

The spermatogonial plate contains 15 chromosomes, two larger than the others ( $a_1$  and  $a_2$ , Fig. 186). These are represented by *a* in the first spermatocyte (Fig. 188a) and also in the second spermatocytes (Figs. 191 and 192, *a*). The growth period shows the odd chromosome (*x*) as a round body with even contour (Fig. 187). The univalent chromosome *x* has the peculiarity here that it never comes to lie in a flat plate with the other chromosomes in the first spermatocyte division, as is indicated in Fig. 189. To get all 8 chromosomes, the equatorial plate must be drawn at two different foci (Figs. 188a and 188b). The odd chromosome always precedes the others to the pole (Fig. 190), never taking the lagging position characteristic of the species previously described. We have noted that this sometimes takes place in other forms (*Vanduzea arcuata*, and the three species of *Ceresa*), but *Phlepsius* is the first form

where this position is invariable. The second spermatocytes contain 8 and 7 chromosomes (Figs. 191 and 192). The spermatids all contain the chromatin nucleolus (Figs. 195, *n*, and 196, *n*) and half of them, an odd chromosome (Figs. 195 and 196, *x*).

#### *Agallia sanguinolenta*

No spermatogonial plates were found in this form. The odd chromosome appears as usual in the growth period (Fig. 197). There are 11 chromosomes in the first spermatocyte (Fig. 198), and 11 and 10 in the second (Figs. 200 and 201). The odd chromosome does not divide in the first spermatocyte metakinesis (Fig. 199), but passes to one pole after the other chromosomes in anaphase (Fig. 206). The spermatids all contain a chromatin nucleolus, and half of them, the odd chromosome (Figs. 203 and 204). Figs. 205 to 207 are drawn from aceto-carmine preparations at the same magnification as Figs. 197 to 204.

#### *Cercopidae*

The testes of the Cercopidae are situated near the posterior end of the abdomen. They are white in color, and each follicle is round, with a comparatively long duct joining it to the vas deferens. The material comprises four species, and the spermatogenesis of none of them resembles very closely that of the species studied by Stevens ('06b).

#### *Clastoptera obtusa*

This species was found on the alder at Cold Spring Harbor. The resting spermatogonium stains very lightly and has a plasmosome (Fig. 208). In preparing for division, the chromatin forms a spireme, which becomes more dense, and then segments (Fig. 209). There are 15 chromosomes in the spermatogonial equatorial plate, all of about the same size (Fig. 210). The division is longitudinal as usual (Figs. 211 and 212). After the telophase, the chromosomes soon become joined by linin connections (Fig. 213), form a compact spireme in early synapsis (Fig. 214), a dense mass in the contraction stage (Fig. 215) and a spireme loosely wound on itself in postsynapsis (Fig. 216). The odd chromosome

appears in the contraction stage distinct from the dense chromatin mass, and remains so in postsynapsis and the early growth stage (Fig. 217). It is from the first, a small, ovoid, smooth-contoured body, and still shows clearly when the spireme has segmented and the tetrads are forming (Fig. 218), and when the dumb-bells are formed (Fig. 219). It takes an eccentric position in the equatorial plate of the first spermatocyte (Fig. 220). It does not divide in the first spermatocyte division (Fig. 221), and is the last chromosome to reach the pole in the anaphase (Fig. 222). As there are 7 chromosomes, plus the odd one, in the first spermatocyte, so there are 8 in half the second spermatocytes (Fig. 223), and 7 in the others (Fig. 224). The odd chromosome behaves like the others in the second division (Figs. 225 and 226), and is not distinguishable in the spermatids, all of which have a chromatin nucleolus (Fig. 227). In the development of the spermatid, the chromatin reticulum first becomes massed on the side of the nucleus toward the axial filament (Fig. 228), and then forms a dense U, leaving the rest of the nucleus clear (Fig. 229). The nucleus then elongates, still leaving a clear space toward the apex (Fig. 230). The mature spermatozoon has a solid dense chromatic head (Fig. 231).

#### *Aphrophora quadrangularis*

This species was found on the grass and low bushes in July near Cold Spring Harbor. Originally a small quantity of material was collected and tried in aceto-carmin, as it was supposed to be the same species that Stevens ('06b) had found in Maine and described. But the reduced number of chromosomes proved to be 11 instead of 12, so material was fixed in Gilson and kept to be studied at a convenient time. The material was obtained from two distinct localities, but not kept separate. The sections showed follicles with 11 chromosomes and a few with 12. Whether this difference corresponds with the difference in locality it is unfortunately not possible to say. Another peculiarity is that the form with 12 chromosomes does not resemble, in some of its stages, the form with 11 chromosomes described by Stevens. The most important stages of the form with 11 chromosomes are shown in Figs. 232 to 242. There are 21 spermatogonial chromosomes (Fig. 232) and



11 and 10 second spermatocyte chromosomes (Figs. 238 and 239). The odd chromosome can be traced as an individual as far back as the contraction stage (Figs. 233, *x*, and 234, *x*). A plasmosome (*p*) also appears in the growth period, the thionin clearly bringing out the difference between the two. One of the 11 chromosomes is larger than the others, as is shown in Figs. 235, 238, 239. The odd chromosome does not divide in the first spermatocyte division (Figs. 236 and 237). The spermatids all contain a chromatin nucleolus (Fig. 242). A few stages of an individual with 12 chromosomes are shown in Figs. 243 to 248. This series much more nearly resembles that of the other form from Cold Spring Harbor with 11 chromosomes, than that of the form found in Maine with 12 chromosomes. The Maine form has no contraction stage (Stevens '06b, Figs. 240 to 249), while this form has a distinct one with the odd chromosome and the plasmosome outside of the spireme in the clear part of the nucleus (Fig. 243). The only possible conclusion seems to be that three species (so determined by the differences in spermatogenesis) have been up to this time grouped as one, and all called *Aphrophora quadrangularis*.

#### *Aphrophora 4-notata*

*Aphrophora 4-notata* is interesting especially in connection with *Aphrophora quadrangularis*, as being another case of difference of chromosome number within the same genus. *Aphrophora 4-notata* has 14 chromosomes for the reduced number (Fig. 250) and consequently 14 in half of the second spermatocytes (Fig. 253) and 13 in the other half (Fig. 254). The odd chromosome is present in the spireme stage (Fig. 249), and does not divide in the first spermatocyte division (Figs. 251 and 252).

#### *Fulgoridæ*

The testes of the *Fulgoridæ* are orange-colored and show through the thin white walls of the abdomen. The separate follicles are oblong. Of the four species in my material, three belong to the genus *Pæciloptera*, and one to the *Amphiscepa*, but according to the spermatogenesis, *P. bivittata* is much more like the *Amphis-*

cepa than like the other two species of *Pæcilopectera*. *P. septentrionalis* and *P. pruinosa* were found on the nettle and the other two species came from sweeping low grasses. In this material, the cells and chromosomes are large and the achromatic structures especially well preserved. The material fixed in Flemming, and stained in thionin makes some of the clearest preparations included in this study.

#### *Pæcilopectera septentrionalis*

The resting spermatogonia of this form are small and stain lightly (Fig. 256). In preparation for division, a spireme is formed, each granule of which splits longitudinally (Fig. 257). The chromatic part of the spireme segments, retaining the linin connections and also an indication of the longitudinal split (Fig. 258). There are 27 chromosomes in the spermatogonial plate, two longer than the others ( $a_1$  and  $a_2$  of Fig. 259). Fig. 260 shows distinctly that this division follows the preliminary longitudinal split. After the telophase, the chromosomes become more diffuse and join into a spireme (Fig. 262). This spireme contracts into a small dense ball at one side of the nucleus (Fig. 263), and then the cell goes through a long growth period in which the diameter is at least doubled. The odd chromosome appears as soon as the spireme becomes pale enough to conceal it no longer (Fig. 264). Then a pair of *m*-chromosomes appears and a small plasmosome (Fig. 265). The plasmosome and odd chromosome both increase in size, the latter having a vacuole in the center (Fig. 266). The odd chromosome has now attained its full size, but while the cell and nucleus continue to increase, the plasmosome keeps on growing (Fig. 267). Even though it is now larger than the odd chromosome, it stains scarcely at all, while the odd chromosome and the *m*-chromosomes stain a deep blue, thus demonstrating the valuable differentiating powers of thionin. In the next stage (Fig. 268) the odd chromosome and the plasmosome are unchanged, but the spireme stains more deeply and shows a longitudinal split. The *m*-chromosomes no longer appear, they have probably become indistinguishable from the other spireme segments. The plasmosome and odd chromosome still keep the same relative size in the pro-

phase, while the tetrads are forming (Fig. 269), the plasmosome sometimes not being dissolved until after the spindle is formed (Fig. 271). There are 14 chromosomes in the equatorial plate of the first spermatocyte (Fig. 270), one of them being marked by its eccentric position, another by its large size. This large chromosome keeps its individuality in all the second spermatocytes, those with 14 chromosomes (Fig. 273), and those with 13 (Fig. 274). The odd chromosome does not divide in the first spermatocyte division (Figs. 271 and 272), but does in the second (Figs. 275 and 276). The development of the spermatid in this family is very peculiar. The nucleus stains quite deeply, so that nothing more can be made out than that there seems to be one condensed body in each spermatid (Fig. 279a). The "Nebenkern" goes through a complicated development somewhat similar at first to that described by Baumgartner ('02). First delicate fibers are formed in it (Fig. 277), then it appears as a long coiled fiber in a clear space, surrounded by a definite membrane (Fig. 278). This space becomes separated by a partition into two tubes, each containing several shorter fibers (Figs. 279a and b). These tubes and fibers both become elongated (Fig. 280). The tubes grow still longer and smaller in diameter, and at the same time twist around each other in an irregular spiral (Fig. 281a). Cross sections through different portions of these twisted tubes indicate that they must also be constricted in places (Fig. 281b). They finally become flattened, presenting some such an appearance as in Fig. 282a, and in cross section as in Fig. 282b. In this species, the chromosomes in the female somatic cells could be counted, and proved to be 28 in number (Fig. 283), there being the same two long ones that appeared in the spermatogonial plate. The significance of the even number in the female, and the odd number in the male will be pointed out in the theoretical considerations.

#### *Pœcilopectera pruinosa*

*Pœcilopectera pruinosa* resembles the last described form externally in every character but color, being a grayish purple instead of a pale green. The principal stages are shown in Figs. 284 to 293, the only difference being that there are two large chromosomes

instead of one, in the first spermatocyte equatorial plate (Fig. 285) and also in the second spermatocyte plates (Figs. 288 and 289). The chromatin in the spermatid nucleus does not stain so deeply, and here it can be demonstrated that there is a chromatin nucleus in all of the spermatids (Figs. 292 and 293), and the odd chromosome besides in half of them (Fig. 292). Here also the female somatic chromosome number is 28. Fig. 294 shows some of the chromosomes overlapping each other, but they are really entirely separate from one another, lying at slightly different levels; it is a late prophase stage of an egg follicle cell before the chromosomes are drawn completely into one plane.

#### *Amphiscepa bivittata*

All this material came from larvæ. The different stages are shown in Figs. 295 to 304. The spermatogonial plates contain 25 chromosomes, two pairs of long ones, one pair longer than the other (Fig. 295). In the rest stage, there are no *m*-chromosomes, but two plasmosomes are present (Fig. 296). The first spermatocyte plate shows two large chromosomes, one larger than the other (Fig. 297), corresponding to the two large pairs of the spermatogonium. The plasmosomes here persist into the metaphase (Fig. 298). The odd chromosome is quite small (Figs. 297,  $\times$ , 298,  $\times$ , 299,  $\times$ ) and does not divide in the first division. Chromosomes *a* and *b* of the first spermatocyte retain their relative sizes in the second spermatocytes, both those containing 13 chromosomes (Fig. 300), and those with 12 (Fig. 301).

#### *Pæciloptera bivittata*

*Pæciloptera bivittata* very closely resembles the last described species, even to the number and relative sizes of its chromosomes (Figs. 305–313). It has two plasmosomes in the growth period, and one or both of these persist in a most remarkable fashion even to the anaphase of the second spermatocyte division (Fig. 312). The size of the chromosomes and cells is greater than in *Amphiscepa bivittata*.

## THEORETICAL CONSIDERATIONS

*Individuality of the Chromosomes*

The theory of the individuality of the chromosomes was first proposed by Boveri ('88) as a result of his work on *Ascaris*. He found a constant number of chromosomes in each species, always half this number in the two maturation divisions, and the original number restored by fertilization. Every year adds to the number of species found conforming to these rules, and consequently making Boveri's theory more plausible. Beginning with Sutton's work in 1900, many species have been shown to give evidence of a more direct nature, and among these, the Hemiptera Homoptera can be classed. In the first place, it is a sign of individuality, when we are able to pick out one chromosome in every equatorial plate by some characteristic size, shape or position. This can be done for 14 out of the 22 species of Hemiptera Homoptera studied, the characteristic usually being the large size of the chromosome (see *Pæcilopectera septentrionalis*, Figs. 270, 273, 274). Secondly, all evidence that supports Montgomery's hypothesis of the union of paternal and maternal chromosomes in synapsis necessarily supports the theory of the individuality of the chromosomes. In *Pæcilopectera septentrionalis*, the large chromosome in the spermatocytes (Fig. 270) is represented in the spermatogonia (Fig. 259) by two large chromosomes. Half of the chromosomes in each spermatogonial plate must have come originally from the spermatozoön, and half from the egg. Only one large chromosome could be received from the spermatozoön, according to Fig. 270, therefore the other large one must have come from the egg. As these two large chromosomes, one paternal and one maternal, are represented by a single chromatic element in the spermatocyte, this must be formed by the union of a paternal with a maternal chromosome of the spermatogonium. Thus we see that the Hemiptera Homoptera are in accord with Montgomery's hypothesis of synapsis and reduction. In the third place, the behavior of the odd chromosome supports Boveri's theory. In the Hemiptera Homoptera, the odd chromosome can seldom be identified in the spermatogonia, but from the contraction stage to the

anaphase of the first spermatocyte, and sometimes to the metaphase of the second spermatocyte (Figs. 56 and 149) its individuality is marked. It takes the basic stains when the rest of the chromatin takes acid stains; it frequently has a smooth round contour in the early prophase, when the other chromosomes are irregular rods or tetrads; it usually is closely applied to the nuclear membrane until that is dissolved, and then keeps an eccentric position in the first spermatocyte equatorial plate; it does not divide in this division, and either precedes or follows the other chromosomes to the pole. In *Vanduzee arcuata* (Fig. 56), where it is intermediate in size, and in *Chlorotetrix* (Fig. 149), where it is the smallest chromosome, its individuality is still marked in the second spermatocyte. Finally the facts that have brought about the dropping of the old discussion about prereduction and postreduction, speak for the individuality of the chromosomes, in that they show the essential point of reduction to be the separation of each maternal chromosome from its paternal mate, and their distribution to different spermatozoa. The uselessness of insisting on prereduction or postreduction is shown within the order Hemiptera, where the odd chromosome may divide in either division; in the Heteroptera, it usually divides in the first, while in the Homoptera, the usual place of division is the second spermatocyte, but *Archimerus* and *Banassa* are exceptions in the former and *Enchenopa* in the latter.

#### *Value of the Number of Chromosomes in Taxonomy and Evolution*

McClung ('05) states that for Orthoptera, a certain number of chromosomes is characteristic for each family, the chromosome grouping marking the genus, and the relative size of the chromosomes indicating the species. Unfortunately this is not true for the Hemiptera Homoptera as the number varies within the family and even within the genus, being constant for the species only. The case of *Aphrophora quadrangularis* may make this doubtful, although it seems more probable that two or three species have previously been included under one name, than that in the same species, the reduced number should be sometimes 12 and sometimes 11, which would not accord with the simplest laws of heredity.

Montgomery has for many years endeavored to determine the stage of evolution by the number of chromosomes that a species possesses, those having few being considered higher in the scale than those with many. The chromatin nucleoli were supposed to be degenerating chromosomes as a species evolves to a higher form. But he has recently collected data from all the scattered literature, tabulated the number of chromosomes and the species, and finds that there is no such correlation ('06b). In the Hemiptera Homoptera there is no reason for considering *Vanduzee arcuata*, with 9 chromosomes, more highly evolved than *Entilia sinuata*, with 11, or *Phlepsius irrotatus*, with 8, more so than *Pæcilopectera septentrionalis*, with 14.

#### *Sex Determination*

We have seen in the historical review of the work on tracheate spermatogenesis, that the most recent and reliable work all points to a dimorphism of the spermatozoa in the forms with an odd chromosome or an unequal pair of chromosomes. McClung was the first to suggest that the one characteristic that most generally divides the animal kingdom into two equal classes is sex, and that therefore, the dimorphism of sex and of spermatozoa may be causally connected. There is need of careful statistical work on the proportion of males and females among different species of insects. In general collecting, however, one gets an impression of equality in numbers. McClung's theory was a brilliant guess, which the work of Stevens and Wilson has substantiated.

The Hemiptera Homoptera furnish additional evidence for this theory. Females of many of the species were sectioned for oögonial and somatic equatorial plates. Only two furnished the desired stages, *Pæcilopectera septentrionalis* and *Pæcilopectera pruinosa*. In both the spermatogonial number is 27, the spermatozoa possessing 13 and 14 chromosomes, and the female somatic number is 28. Stevens and Wilson have shown that there is no difference between the somatic number and the unreduced number in the germ cells; in the female, both numbers are even, in the male, both are odd (or even, when a small chromosome is included). As the female somatic number in *Pæcilopectera* is even, the oögonial

number must also be even, and all the matured eggs necessarily possess the same number of chromosomes, 14. Applying Wilson's ('06b) formula for sex determination to the Pœciloptera, we have the following:

I Egg (14 chromosomes) + Spermatozoön (14 chromosomes)  
= Female (28 chromosomes).

II Egg (14 chromosomes) + Spermatozoön (13 chromosomes)  
= Male (27 chromosomes).

Here again it is possible to apply Castle's ('00) theory of sex as a Mendelian character, which has been so fully elaborated and applied to the case of the odd chromosome by Wilson. It involves the assumption of two kinds of eggs, male and female, as well as the two kinds of spermatozoa which are actually to be observed. It also involves the assumption of selective fertilization: an egg bearing the female determinant must be fertilized by a spermatozoön with the male determinant, while an egg bearing the male determinant must be fertilized by a spermatozoön with the female determinant. In case II of the above formula when the egg is fertilized by the spermatozoön without the odd chromosome, the sex determinant must be introduced by the egg; and as in this case, a male is produced, the eggs fertilized by a spermatozoön without an odd chromosome must bear the male determinant, and the chromosome which has disappeared in the males must be the one with the female character. So in case I, where the egg is fertilized by the spermatozoön with the odd chromosome, the spermatozoön must bear the male character and the egg the female; as this combination always results in a female, it is necessary to assume that the male character is recessive and the female dominant. The above formulæ can be extended to show these assumptions and will read thus:

I ♀ Egg (14 chromosomes) + (♂) Spermatozoön (14 chromosomes) = ♀ (♂) Female (28 chromosomes).

II (♂) Egg (14 chromosomes) + (o) Spermatozoön (13 chromosomes) = (♂) (o) Male (27 chromosomes).

This is the part of Wilson's theory that deals with the case presented by Pœciloptera and presumably the other Hemiptera Homoptera. The facts as far as they go are not at variance with the theory.



## SUMMARY

1 An odd chromosome is present in the spermatogenesis of 22 species of the Hemiptera Homoptera, as shown in each case by some or all of the following facts:

*a* The spermatogonia have an uneven number of chromosomes.  
*b* A dense body takes basic stains in the growth period.  
*c* One chromosome stands in an eccentric position in the first spermatocyte equatorial plate.

*d* In the metaphase of the first spermatocyte division, one chromosome does not divide, and has half the valence of the others, as shown by its spherical shape when the others are like dumb-bells.

*e* In anaphase of the first spermatocyte division, one chromosome at one pole behaves differently from the others, either preceding or lagging behind.

*f* Half of the equatorial plates of the second spermatocytes contain the same number of chromosomes as those of the first spermatocytes, but half contain one less.

*g* Half of the spermatids contain a condensed body, taking basic stains, which is the odd chromosome.

2 The odd chromosome shows certain variations in behavior, either individual or specific.

*a* In the anaphase of the division where it does not divide, in some cells it may precede the other chromosomes to the poles, while in others it lags behind them.

This individual variation is a characteristic of certain species, the three species of *Ceresa* and *Vanduzea arcuata*, while most of the species studied have the odd chromosome always lagging behind, and *Phlepsius irrotatus* has it always preceding the others.

*b* In *Enchenopa binotata*, it divides in the first division, and in the second division, where it does not divide, it neither precedes nor lags behind the others.

*c* The shape of the odd chromosome in the growth period varies. It may be always spherical or ovoid with a smooth contour, as in the *Fulgoridæ*, *Cercopidæ*, *Jassidæ*, and some of the *Membracidæ*. It may be long and uneven in contour as in *Vanduzea arcuata* and *Enchenopa binotata*.

It may pass through both forms in different stages, as in *Entilia sinuata*.

3 In the spermatids of 19 species; that is, all except three of the Membracidæ, there is a chromatin nucleolus in all of the spermatids entirely independent of the odd chromosome. In seven of these species, the odd chromosome is present also in half of the spermatids, in others there is no indication of it. In the three Membracidæ without the chromatin nucleolus, *Entilia sinuata*, *Vanduzea arcuata*, and *Campylenchia curvata*, the odd chromosome is present in half of the spermatids.

4 In the genus *Ceresa*, in the contraction stage some of the basichromatin is thrown out from the chromatin loops and persists through the growth period as a chromatin deposition on the nuclear membrane and finally dissolves without apparently taking part in the formation of the chromosomes for the first spermatocyte division.

5 In three species, *Campylenchia curvata*, *Pœcilopectera septentrionalis*, and *Pœcilopectera pruinosa*, a pair of *m*-chromosomes remain condensed in the growth period.

6 The number of chromosomes has no significance for grouping species into families. In reduced number,

in the Membracidæ, 5 species have 11 chromosomes
2 species have 10 chromosomes
1 species has 9 chromosomes

in the Jassidæ, 2 species have 12 chromosomes
2 species have 11 chromosomes
1 species has 9 chromosomes
1 species has 8 chromosomes

in the Cercopidæ, 1 species has 14 chromosomes
1 species has 12 chromosomes
1 species has 11 chromosomes
1 species has 8 chromosomes

in the Fulgoridæ, 2 species have 14 chromosomes
2 species have 13 chromosomes

7 The number of chromosomes has no significance for grouping species into genera.

<i>Chlorotetrix unicolor</i> ,	11 chromosomes
<i>Chlorotetrix vividus</i> ,	9 chromosomes
<i>Aphrophora quadrangularis</i> ,	11 or 12 chromosomes
<i>Aphrophora 4-notata</i> ,	14 chromosomes
<i>Pœcilopectera septentrionalis</i> ,	14 chromosomes
<i>Pœcilopectera bivittata</i> ,	13 chromosomes

8 The number of chromosomes is constant for each species. In the case of *Aphrophora quadrangularis*, where there have been found both 11 and 12 chromosomes, probably two species are present, which have not been separated in classification.

9 The only points in the spermatogenesis in which all of the species of one family resemble each other more closely than they do the species of the other families are the appearance of some of the growth stages and the transformation of the spermatid into the spermatozoon.

10 In fourteen of the species studied, the individuality of certain chromosomes can be traced from the spermatogonium to the second spermatocyte, a pair of similar chromosomes in the spermatogonium bearing the same size relation to the other chromosomes of the equatorial plate as a single chromosome bears to the others in the first and second spermatocyte plates. In all the species, the odd chromosome can be traced as keeping its individuality from the growth period to the anaphase of the first spermatocyte division, in *Chlorotetrix* and *Vanduzeeia arcuata* to the metaphase of the second spermatocyte division, and in *Enchenopa binotata*, from the spermatogonial plate to the telophase of the second spermatocyte division.

11 In all 22 species, there is a dimorphism of the spermatozoa, which probably corresponds to the natural dimorphism of sex.

12 Two species of *Fulgoridæ* in which the female somatic number of chromosomes is 28, while the spermatogonial number is 27, furnish further proof for the theory of sex determination advanced by McClung, Wilson and Stevens.

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### DESCRIPTION OF PLATES.

The figures were drawn with the aid of the Zeiss-Abbe drawing camera, No. 111. Figs. 1-46 were drawn with a Leitz oil immersion obj.  $\frac{1}{12}$  and a Zeiss compensating oc. 12, Figs. 47-313 with a Zeiss apochromatic oil immersion obj. 2 mm., oc. 12. They have been reduced one-third, giving a magnification of about 1000 diameters.

#### *Abbreviations Used on Plates*

$a_1$  and  $a_2$  = one pair of spermatogonial chromosomes.

$a$  = a bivalent primary spermatocyte chromosome representing  $a_1$  and  $a_2$ .

$b_1$  and  $b_2$  = one pair of spermatogonial chromosomes.

$b$  = a bivalent primary spermatocyte chromosome representing  $b_1$  and  $b_2$ .

$m_1$  and  $m_2$  = a pair of  $m$ -chromosomes.

$n$  = chromatin nucleolus.

$p$ ,  $p_1$ ,  $p_2$  = plasmosomes.

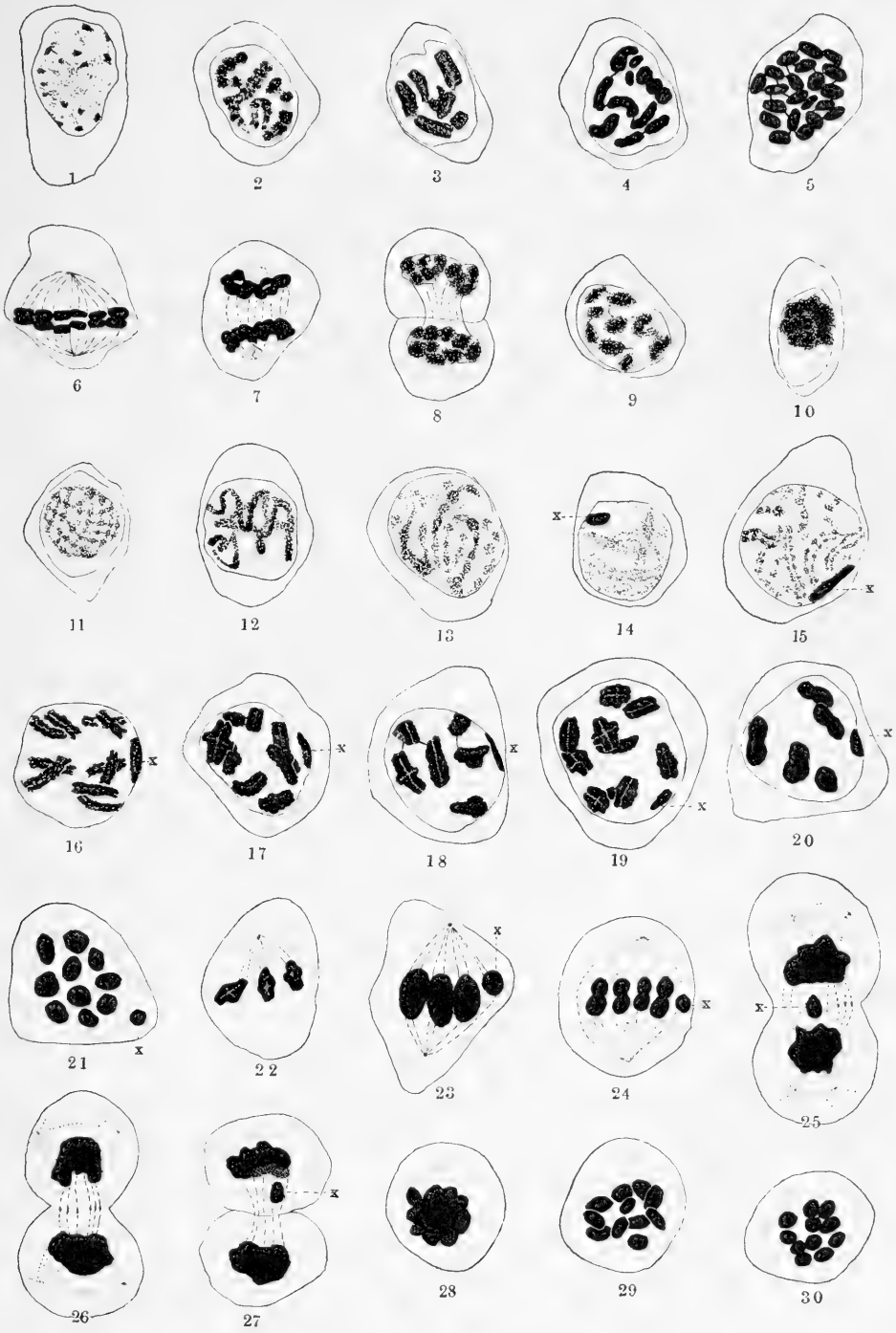
$x$  = odd chromosome.



PLATE I

*Entilia sinuata* (Family Membracidae)

- Fig. 1 Spermatogonial rest stage.
- Fig. 2 Spermatogonial spireme.
- Fig. 3 Spermatogonium, segmentation of the spireme, each segment longitudinally split.
- Fig. 4 Spermatogonium, condensation of the segments of the spireme.
- Fig. 5 Spermatogonial equatorial plate, 21 chromosomes.
- Fig. 6 Spermatogonial metaphase.
- Fig. 7 Spermatogonial anaphase.
- Fig. 8 Spermatogonial telophase, formation of nuclear membrane.
- Fig. 9 Spermatogonial telophase, polar view.
- Fig. 10 First spermatocyte, contraction stage.
- Fig. 11 First spermatocyte, early postsynapsis stage.
- Fig. 12 First spermatocyte, late postsynapsis, fine spireme.
- Fig. 13 First spermatocyte, coarse spireme.
- Fig. 14 First spermatocyte, rest stage.
- Fig. 15 First spermatocyte, split spireme.
- Fig. 16 First spermatocyte, spireme divided into 11 split segments.
- Figs. 17-19 First spermatocyte, early prophase, tetrad formation.
- Fig. 20 First spermatocyte, late prophase, dumb-bell formation.
- Fig. 21 First spermatocyte, equatorial plate, 11 chromosomes.
- Fig. 22 First spermatocyte, metaphase, chromosomes still tetrads.
- Figs. 23, 24 First spermatocyte, metaphase.
- Figs. 25, 26 First spermatocyte, anaphase, centrosomes divided for the second division.
- Fig. 27 First spermatocyte, telophase.
- Fig. 28 First spermatocyte, telophase, polar view.
- Fig. 29 Rearrangement of chromosomes for the second spermatocyte division.
- Fig. 30 Second spermatocyte, equatorial plate, 11 chromosomes.



MEMBRACIDÆ

A. M. B. *del.*

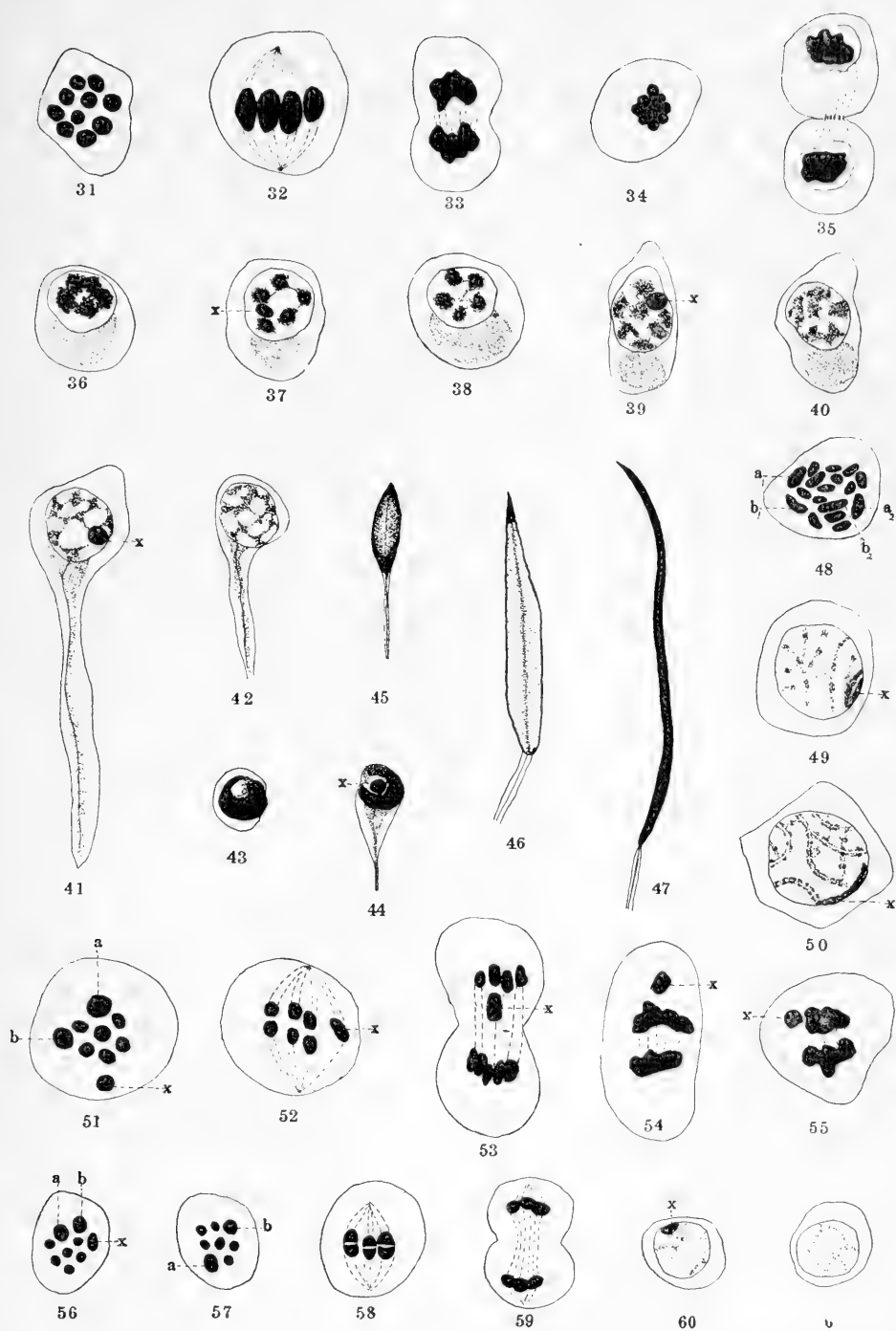
PLATE II

*Entilia sinuata* (continued)

- Fig. 31 Second spermatocyte, equatorial plate, 10 chromosomes.  
Fig. 32 Second spermatocyte, metaphase.  
Fig. 33 Second spermatocyte, anaphase.  
Fig. 34 Second spermatocyte, anaphase, polar view.  
Fig. 35 Second spermatocyte, telophase.  
Fig. 36 Spermatid, first stage.  
Figs. 37, 38 Spermatids, second stage, half with  $x$ , half without.  
Figs. 39, 40 Spermatids, third stage, half contain  $x$ , half do not.  
Figs. 41, 42 Spermatids, formation of axial filament, half contain  $x$ , half do not.  
Figs. 43, 44 Spermatids, condensation of the chromatin, half contain  $x$ , half do not.  
Figs. 45, 46 Spermatids, later stages.  
Fig. 47 Mature spermatozoon.

*Vanduzee arcuata* (Family Membracidae)

- Fig. 48 Spermatogonial equatorial plate, 17 chromosomes.  
Figs. 49, 50 First spermatocyte, growth period.  
Fig. 51 First spermatocyte, equatorial plate, 9 chromosomes.  
Fig. 52 First spermatocyte, metaphase.  
Figs. 53-55 First spermatocyte, anaphase.  
Figs. 56, 57 Second spermatocytes, equatorial plates, containing 9 and 8 chromosomes, respectively.  
Fig. 58 Second spermatocyte, metaphase.  
Fig. 59 Second spermatocyte, anaphase.  
Figs. 60, 61 Spermatids, half contain  $x$ , half do not.



MEMBRACIDÆ

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### PLATE III

#### *Ceresa taurina* (Family Membracidae)

Figs. 62, 63 First spermatocyte contraction stage, a mass of rejected basichromatin at the base of the loops.

Figs. 64-66 First spermatocyte, rest stage, showing rejected basichromatin.

Fig. 67 First spermatocyte, rest stage, showing  $\alpha$  in the midst of the rejected chromatin.

Fig. 68 First spermatocyte, split spireme stage. Most of the rejected chromatin has dissolved, showing  $\alpha$  plainly.

Fig. 69 First spermatocyte, prophase.

Fig. 70 First spermatocyte, equatorial plate, 11 chromosomes.

Figs. 71-73 First spermatocyte, metaphase.

Figs. 74, 75 First spermatocyte, anaphase.

Figs. 76, 77 Second spermatocyte, equatorial plates, containing 11 and 10 chromosomes, respectively.

Fig. 78 Second spermatocyte, metaphase.

Fig. 79 Second spermatocyte, anaphase.

Fig. 80 Spermatid, with chromatin nucleolus.

#### *Ceresa bubalus* (Family Membracidae)

Fig. 81 First spermatocyte, synapsis stage, showing rejected chromatin.

Fig. 82 First spermatocyte, rest stage, showing rejected chromatin.

Fig. 83 First spermatocyte, equatorial plate, 11 chromosomes.

Figs. 84, 85 First spermatocytes, metaphase.

Figs. 86, 87 First spermatocytes, anaphase.

Figs. 88, 89 Second spermatocytes, equatorial plates, containing 11 and 10 chromosomes, respectively.

Fig. 90 Second spermatocyte, metaphase.

Fig. 91 Second spermatocyte, anaphase.

Fig. 92 Spermatid, with chromatin nucleolus.

#### *Ceresa diceros* (Family Membracidae)

Fig. 93 First spermatocyte, rest stage, showing rejected chromatin.

Fig. 94 First spermatocyte, equatorial plate, 11 chromosomes.

Fig. 95 First spermatocyte, metaphase.

Fig. 96 First spermatocyte, anaphase.

Figs. 97, 98 Second spermatocytes, equatorial plates, containing 11 and 10 chromosomes, respectively.

Fig. 99 Second spermatocyte, metaphase.

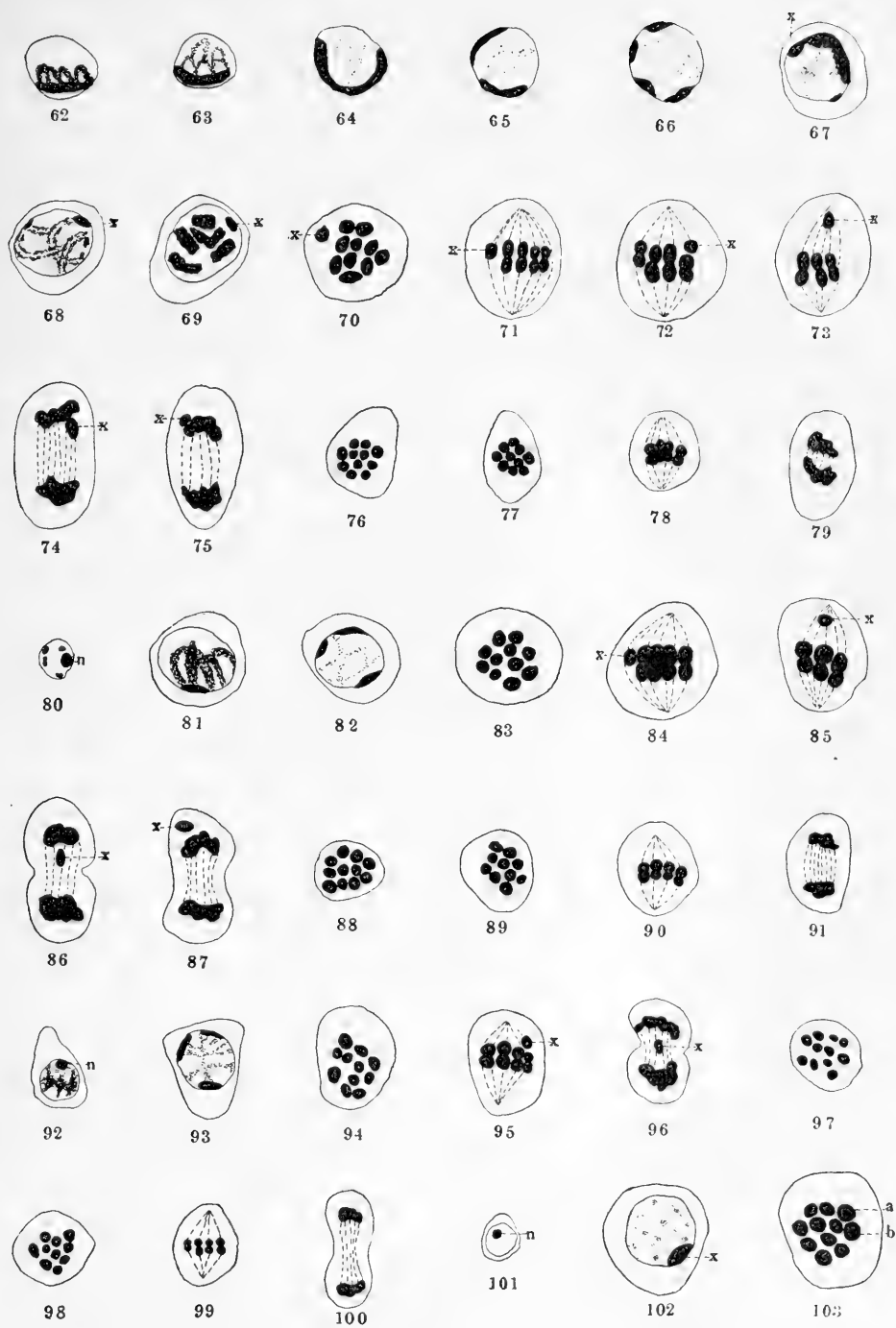
Fig. 100 Second spermatocyte, anaphase.

Fig. 101 Spermatid with chromatin nucleolus.

#### *Atymna castanea* (Family Membracidae)

Fig. 102 First spermatocyte, rest stage.

Fig. 103 First spermatocyte, equatorial plate, 11 chromosomes.



MEMBRACIDÆ

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PLATE IV

*Atymna castanea* (continued)

- Figs. 104, 105 First spermatocyte, metaphase.  
Fig. 106 First spermatocyte, anaphase.  
Figs. 107, 108 Second spermatocytes, equatorial plates, containing 11 and 10 chromosomes, respectively.  
Fig. 109 Second spermatocyte, metaphase.  
Fig. 110 Second spermatocyte, anaphase.  
Fig. 111 Spermatid, with chromatin nucleolus.

*Campylenchia curvata* (Family Membracidae)

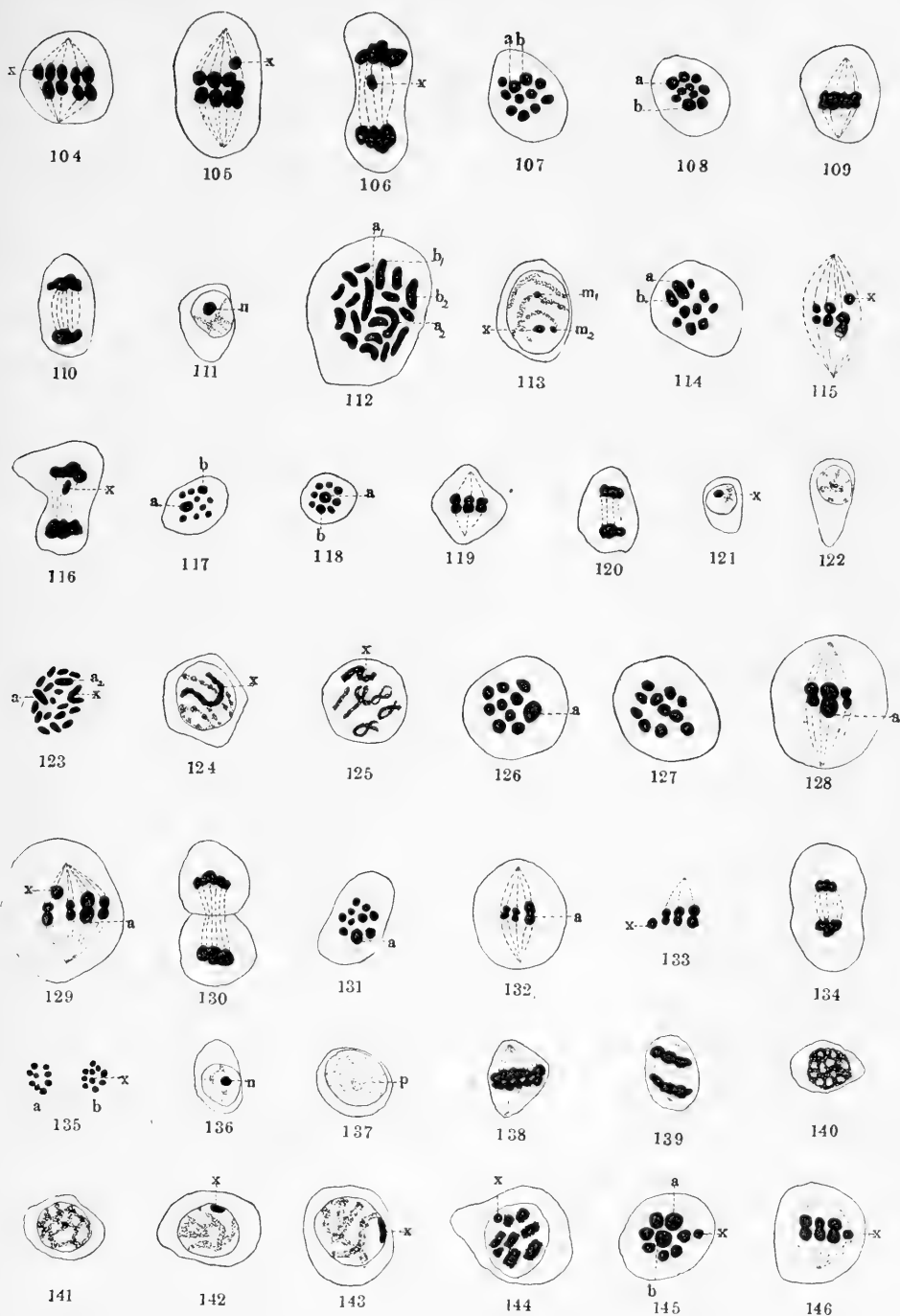
- Fig. 112 Spermatogonial equatorial plate, 19 chromosomes.  
Fig. 113 First spermatocyte, rest stage.  
Fig. 114 First spermatocyte, equatorial plate, 10 chromosomes.  
Fig. 115 First spermatocyte, metaphase.  
Fig. 116 First spermatocyte, anaphase.  
Figs. 117, 118 Second spermatocytes, equatorial plates, containing 10 and 9 chromosomes, respectively.  
Fig. 119 Second spermatocyte, metaphase.  
Fig. 120 Second spermatocyte, anaphase.  
Figs. 121, 122 Spermatids, half with  $\alpha$ , half without.

*Enchenopa binotata* (Family Membracidae)

- Fig. 123 Spermatogonial equatorial plate, 19 chromosomes.  
Fig. 124 First spermatocyte, spireme stage.  
Fig. 125 First spermatocyte, early prophase.  
Fig. 126 First spermatocyte, equatorial plate, 10 chromosomes.  
Fig. 127 First spermatocyte, equatorial plate, 11 chromosomes, occasionally found.  
Figs. 128, 129 First spermatocytes, metaphase.  
Fig. 130 First spermatocyte, anaphase.  
Fig. 131 Second spermatocyte, equatorial plate, 10 chromosomes.  
Figs. 132, 133 Second spermatocytes, metaphase.  
Fig. 133  $\alpha$  does not divide in this division.  
Fig. 134 Second spermatocyte, anaphase.  
Fig. 135a and b Second spermatocyte anaphase, two plates from the same spindle, 9 chromosomes in one, 10 in the other.  
Fig. 136 Spermatid, with chromatin nucleolus.

*Chlorotettix unicolor* and *Chlorotettix vividus* Family Jassidae

- Fig. 137 Spermatogonial rest stage.  
Fig. 138 Spermatogonial metaphase.  
Fig. 139 Spermatogonial anaphase.  
Fig. 140 First spermatocyte, contraction stage.  
Fig. 141 First spermatocyte, spireme stage.  
Fig. 142 First spermatocyte, rest stage.  
Fig. 143 First spermatocyte, split spireme stage.  
Fig. 144 First spermatocyte, prophase.  
Fig. 145 First spermatocyte, equatorial plate, 9 chromosomes.  
Fig. 146 First spermatocyte, metaphase.



MEMBRACIDÆ AND JASSIDÆ

A. M. B. del.



PLATE V

*Chlorotetrix unicolor* and *Chlorotetrix vividus* (continued)

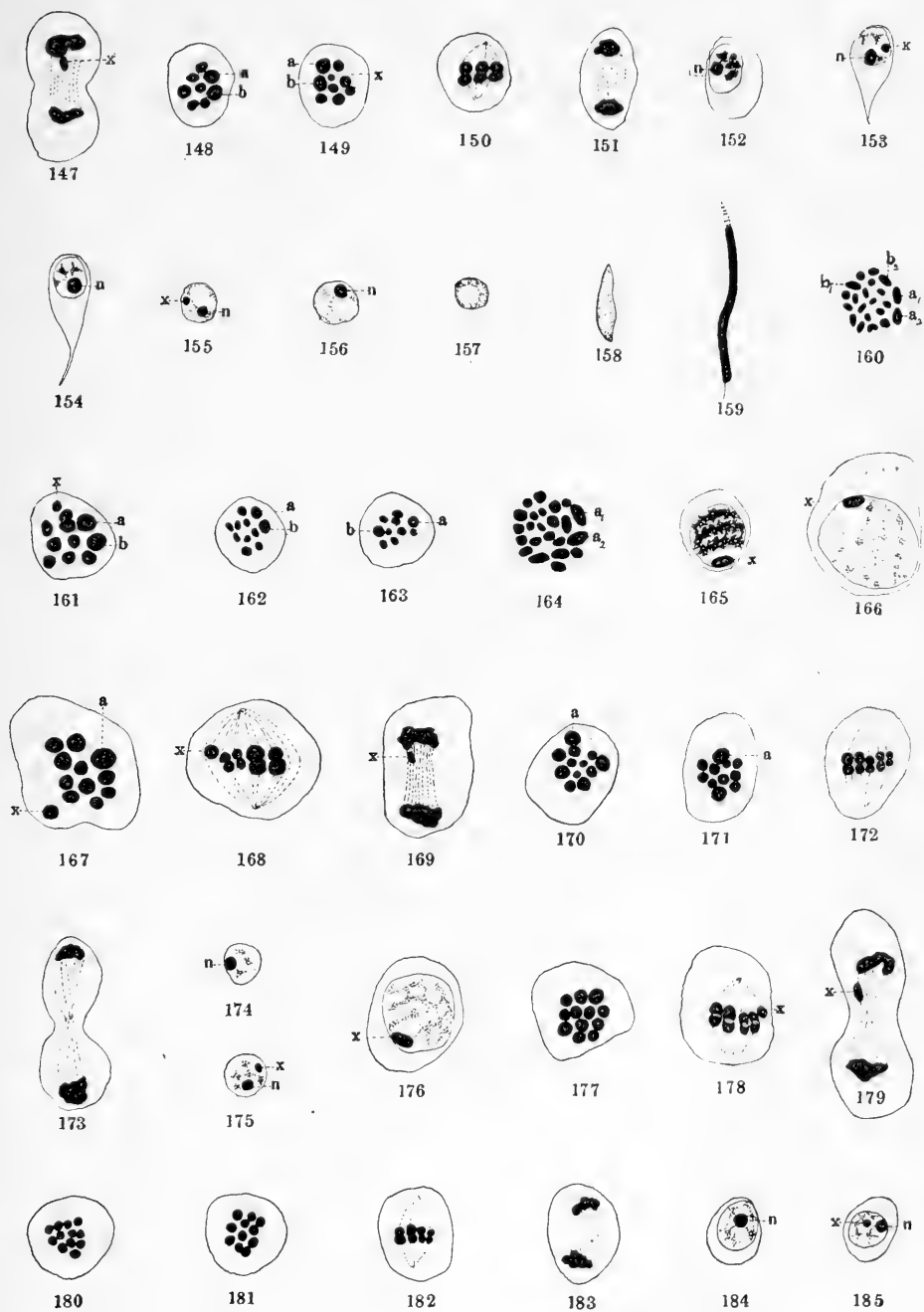
- Fig. 147 First spermatocyte, anaphase.  
Figs. 148, 149 Second spermatocytes, equatorial plates, containing 8 and 9 chromosomes, respectively.  
Fig. 150 Second spermatocyte, metaphase.  
Fig. 151 Second spermatocyte, anaphase.  
Fig. 152 Spermatid, first stage.  
Figs. 153, 154 Spermatid, second stage, half with  $x$ , half without.  
Figs. 155, 156 Spermatid, third stage, half with  $x$ , half without.  
Figs. 157, 158 Late spermatid stages.  
Fig. 159 Head of mature spermatozoön.  
Fig. 160 Spermatogonial equatorial plate, 21 chromosomes.  
Fig. 161 First spermatocyte equatorial plate, 11 chromosomes.  
Figs. 162, 163 Second spermatocytes, equatorial plates, containing 11 and 10 chromosomes, respectively.

*Dicrocephala coccinea* (Family Jasside)

- Fig. 164 Spermatogonial equatorial plate, 23 chromosomes.  
Fig. 165 First spermatocyte, postsynapsis stage.  
Fig. 166 First spermatocyte, rest stage.  
Fig. 167 First spermatocyte, equatorial plate, 12 chromosomes.  
Fig. 168 First spermatocyte, metaphase.  
Fig. 169 First spermatocyte, anaphase.  
Figs. 170, 171 Second spermatocytes, equatorial plates, containing 12 and 11 chromosomes, respectively.  
Fig. 172 Second spermatocyte, metaphase.  
Fig. 173 Second spermatocyte, anaphase.  
Figs. 174, 175 Spermatids, half without  $x$ , half with.

*Dicrocephala mollipes* (Family Jasside)

- Fig. 176 First spermatocyte, rest stage.  
Fig. 177 First spermatocyte, equatorial plate, 12 chromosomes.  
Fig. 178 First spermatocyte, metaphase.  
Fig. 179 First spermatocyte, anaphase.  
Figs. 180, 181 Second spermatocytes, equatorial plates, containing 12 and 11 chromosomes, respectively.  
Fig. 182 Second spermatocyte, metaphase.  
Fig. 183 Second spermatocyte, anaphase.  
Figs. 184, 185 Spermatids, half without  $x$ , half with.



JASSIDÆ

A. M. B. *del.*

PLATE VI

*Phlepsius irrotatus* (Family Jassidæ)

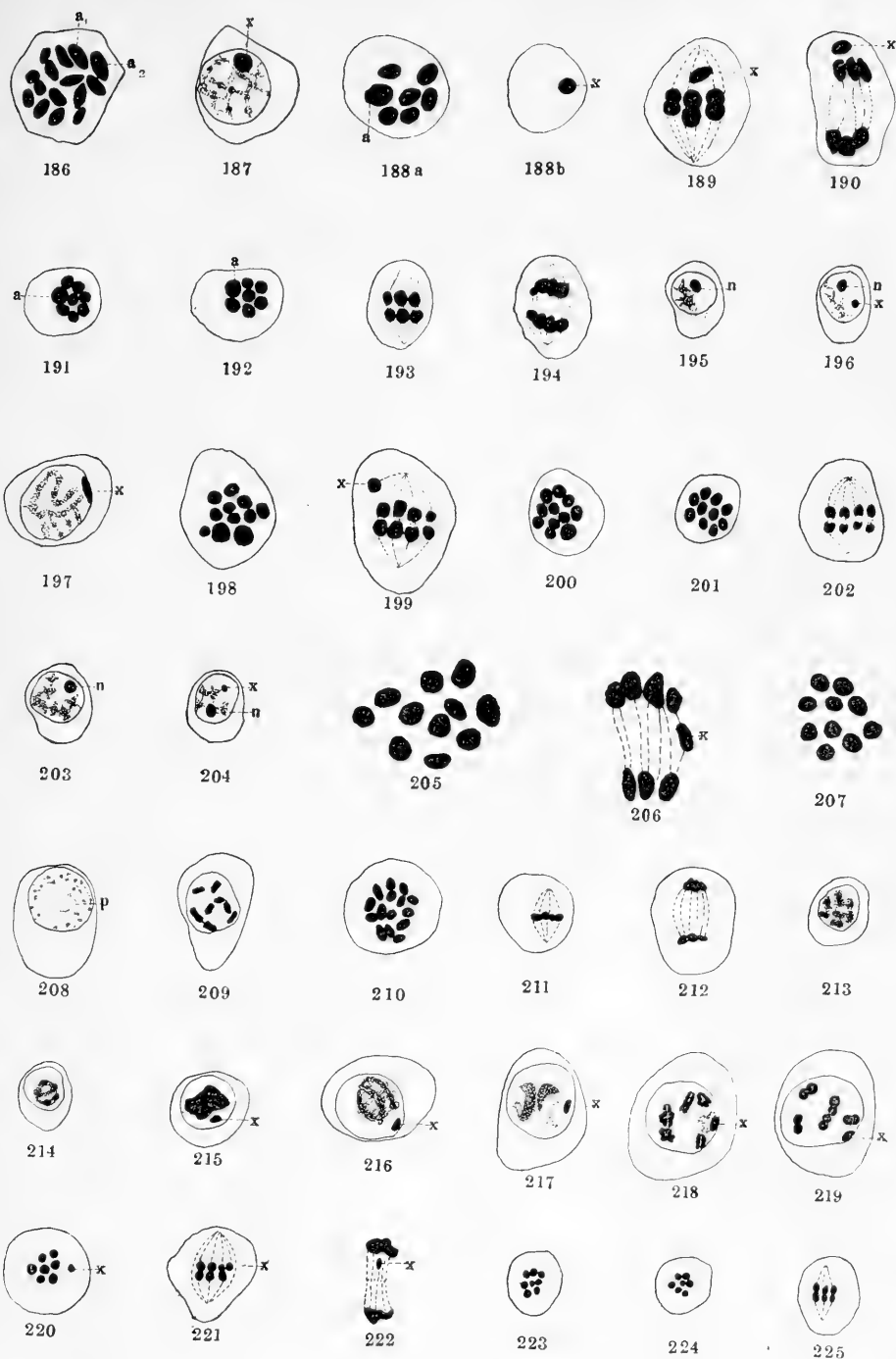
- Fig. 186 Spermatogonial equatorial plate, 15 chromosomes.  
Fig. 187 First spermatocyte, rest stage.  
Fig. 188a and b First spermatocyte, equatorial plate, and the odd chromosome  $x$ .  
Fig. 189 First spermatocyte, metaphase.  
Fig. 190 First spermatocyte anaphase.  
Figs. 191, 192 Second spermatocytes, equatorial plates, containing 8 and 7 chromosomes, respectively.  
Fig. 193 Second spermatocyte, metaphase.  
Fig. 194 Second spermatocyte, anaphase.  
Figs. 195, 196 Spermatids, half without  $x$ , half with.

*Agallia sanguinolenta* (Family Jassidæ)

- Fig. 197 First spermatocyte, spireme stage.  
Fig. 198 First spermatocyte, equatorial plate, 11 chromosomes.  
Fig. 199 First spermatocyte, metaphase.  
Figs. 200, 201 Second spermatocytes, equatorial plates, containing 11 and 10 chromosomes, respectively.  
Fig. 202 Second spermatocyte, early anaphase.  
Figs. 203, 204 Spermatids, half without  $x$ , half with.  
Fig. 205 First spermatocyte, equatorial plate, aceto-carmin preparation.  
Fig. 206 First spermatocyte, anaphase, aceto-carmin preparation.  
Fig. 207 Second spermatocyte, equatorial plate, aceto-carmin preparation.

*Clastoptera obtusa* (Family Cercopidæ)

- Fig. 208 Spermatogonial rest stage.  
Fig. 209 Spermatogonial prophase.  
Fig. 210 Spermatogonial equatorial plate, 15 chromosomes.  
Fig. 211 Spermatogonial metaphase.  
Fig. 212 Spermatogonial anaphase.  
Figs. 213, 214 First spermatocyte, early synapsis.  
Fig. 215 First spermatocyte, contraction stage.  
Fig. 216 First spermatocyte, postsynapsis stage.  
Fig. 217 First spermatocyte, spireme stage.  
Fig. 218 First spermatocyte, early prophase, tetrad formation.  
Fig. 219 First spermatocyte, prophase, dumb-bell formation.  
Fig. 220 First spermatocyte, equatorial plate, 8 chromosomes.  
Fig. 221 First spermatocyte, metaphase.  
Fig. 222 First spermatocyte, anaphase.  
Figs. 223, 224 Second spermatocytes, equatorial plates containing 8 and 7 chromosomes, respectively.  
Fig. 225 Second spermatocyte, metaphase.



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PLATE VII

*Clastoptera obtusa* (Continued)

- Fig. 226 Second spermatocyte, anaphase.  
Fig. 227 Early spermatid, with chromatin nucleolus.  
Fig. 228 Spermatid, formation of axial filament.  
Figs. 229, 230 Later spermatids.  
Fig. 231 Mature spermatozoön.

*Aphrophora quadrangularis* with 11 chromosomes (Family Cercopidae)

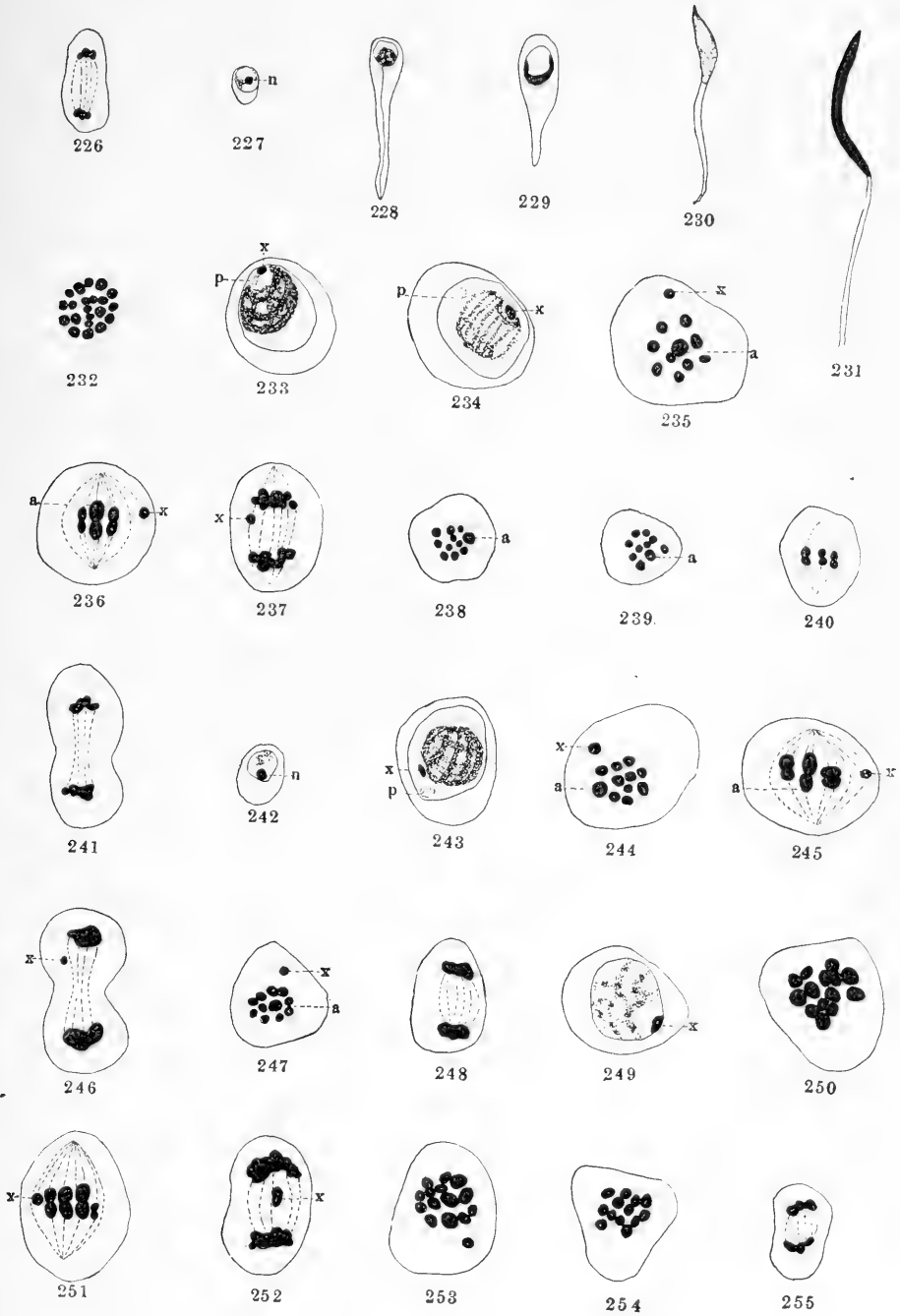
- Fig. 232 Spermatogonial equatorial plate, 21 chromosomes.  
Fig. 233 First spermatocyte, contraction stage.  
Fig. 234 First spermatocyte, spireme stage.  
Fig. 235 First spermatocyte, equatorial plate, 11 chromosomes.  
Fig. 236 First spermatocyte, metaphase.  
Fig. 237 First spermatocyte, anaphase.  
Figs. 238, 239 Second spermatocytes, equatorial plates, containing 11 and 10 chromosomes, respectively.  
Fig. 240 Second spermatocyte, metaphase.  
Fig. 241 Second spermatocyte, anaphase.  
Fig. 242 Spermatid, with chromatin nucleolus.

*Aphrophora quadrangularis* with 12 chromosomes (Family Cercopidae)

- Fig. 243 First spermatocyte, contraction stage.  
Fig. 244 First spermatocyte, equatorial plate, 12 chromosomes.  
Fig. 245 First spermatocyte, metaphase.  
Fig. 246 First spermatocyte, anaphase.  
Fig. 247 Second spermatocyte, equatorial plate, 12 chromosomes.  
Fig. 248 Second spermatocyte, anaphase.

*Aphrophora 4-notata* (Family Cercopidae)

- Fig. 249 First spermatocyte, spireme stage.  
Fig. 250 First spermatocyte, equatorial plate, 14 chromosomes.  
Fig. 251 First spermatocyte, metaphase.  
Fig. 252 First spermatocyte, anaphase.  
Figs. 253, 254 Second spermatocytes, equatorial plates, containing 14 and 13 chromosomes, respectively.  
Fig. 255 Second spermatocyte, anaphase.



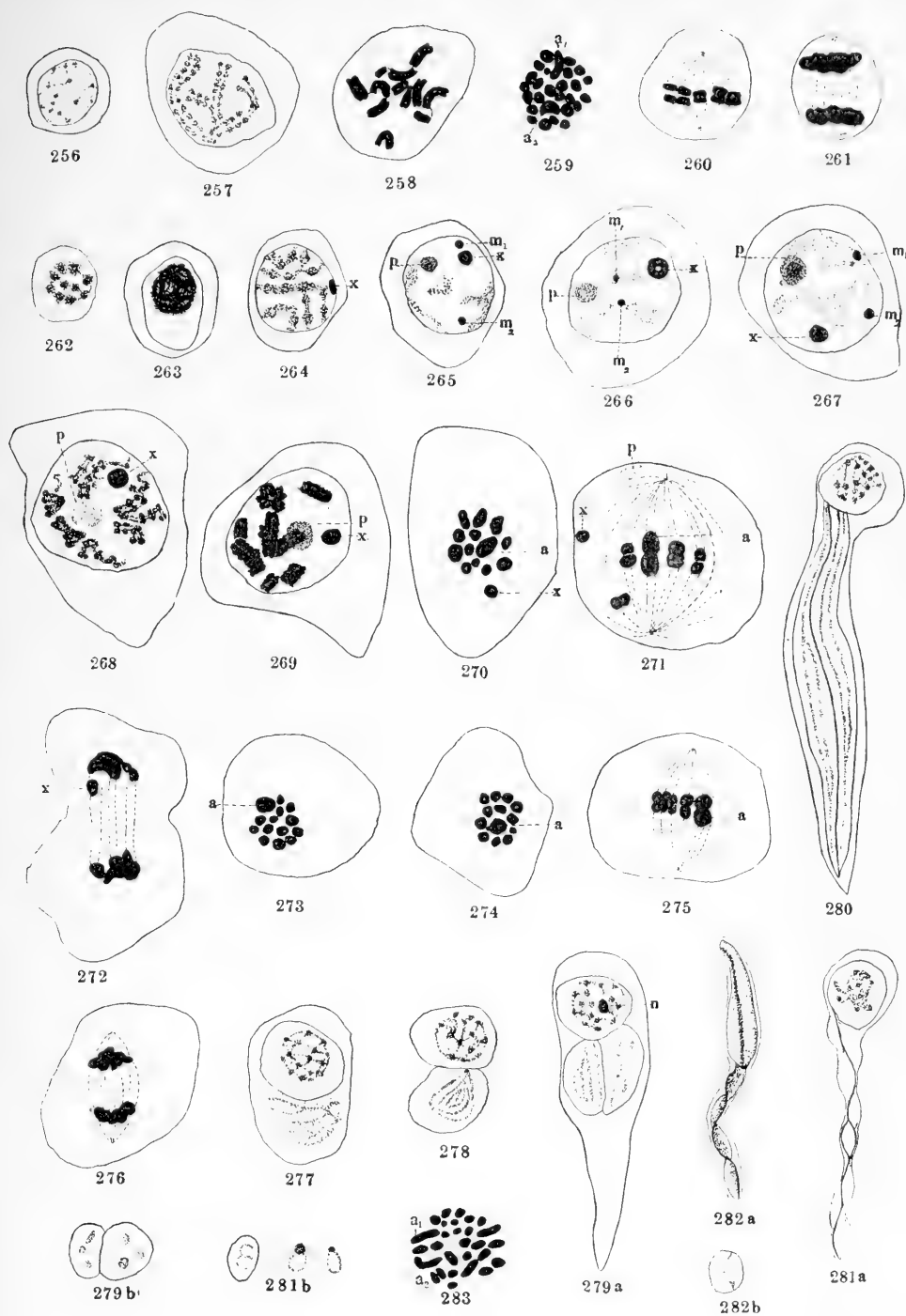
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PLATE VIII

*Pæcilopectera septentrionalis* (Family Fulgoridæ)

- Fig. 256 Spermatogonial rest stage.  
Fig. 257 Spermatogonial split spireme.  
Fig. 258 Spermatogonium, spireme segmented and condensed, segments split.  
Fig. 259 Spermatogonial equatorial plate, 27 chromosomes.  
Fig. 260 Spermatogonial metaphase.  
Fig. 261 Spermatogonial anaphase.  
Fig. 262 First spermatocyte, early synapsis stage.  
Fig. 263 First spermatocyte, contraction stage.  
Fig. 264 First spermatocyte, spireme stage.  
Figs. 265, 267 First spermatocyte, rest stages, growth in size of nucleus and cell.  
Fig. 268 First spermatocyte, split spireme stage.  
Fig. 269 First spermatocyte, prophase, tetrad formation.  
Fig. 270 First spermatocyte, equatorial plate, 14 chromosomes.  
Fig. 271 First spermatocyte, metaphase.  
Fig. 272 First spermatocyte, anaphase.  
Figs. 273, 274 Second spermatocytes, equatorial plates, containing 14 and 13 chromosomes, respectively.  
Fig. 275 Second spermatocyte, metaphase.  
Fig. 276 Second spermatocyte, anaphase.  
Figs. 277, 278 Spermatids, formation of fibers in the "Nebenkern."  
Fig. 279a Spermatid, "Nebenkern" separated by a partition into two tubes.  
Fig. 279b Cross section of "Nebenkern" structure as in 279a.  
Fig. 280 Spermatid, elongation of fibers and tubes.  
Fig. 281a Spermatid, irregular spiral of twisted tubes.  
Fig. 281b Cross sections of tubes of 281a.  
Fig. 282a Spermatid, further twisting and flattening.  
Fig. 282b Cross section of 282a.  
Fig. 283 Female somatic equatorial plate, 28 chromosomes.



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PLATE IX

*Paciloptera pruinosa* (Family Fulgoridæ)

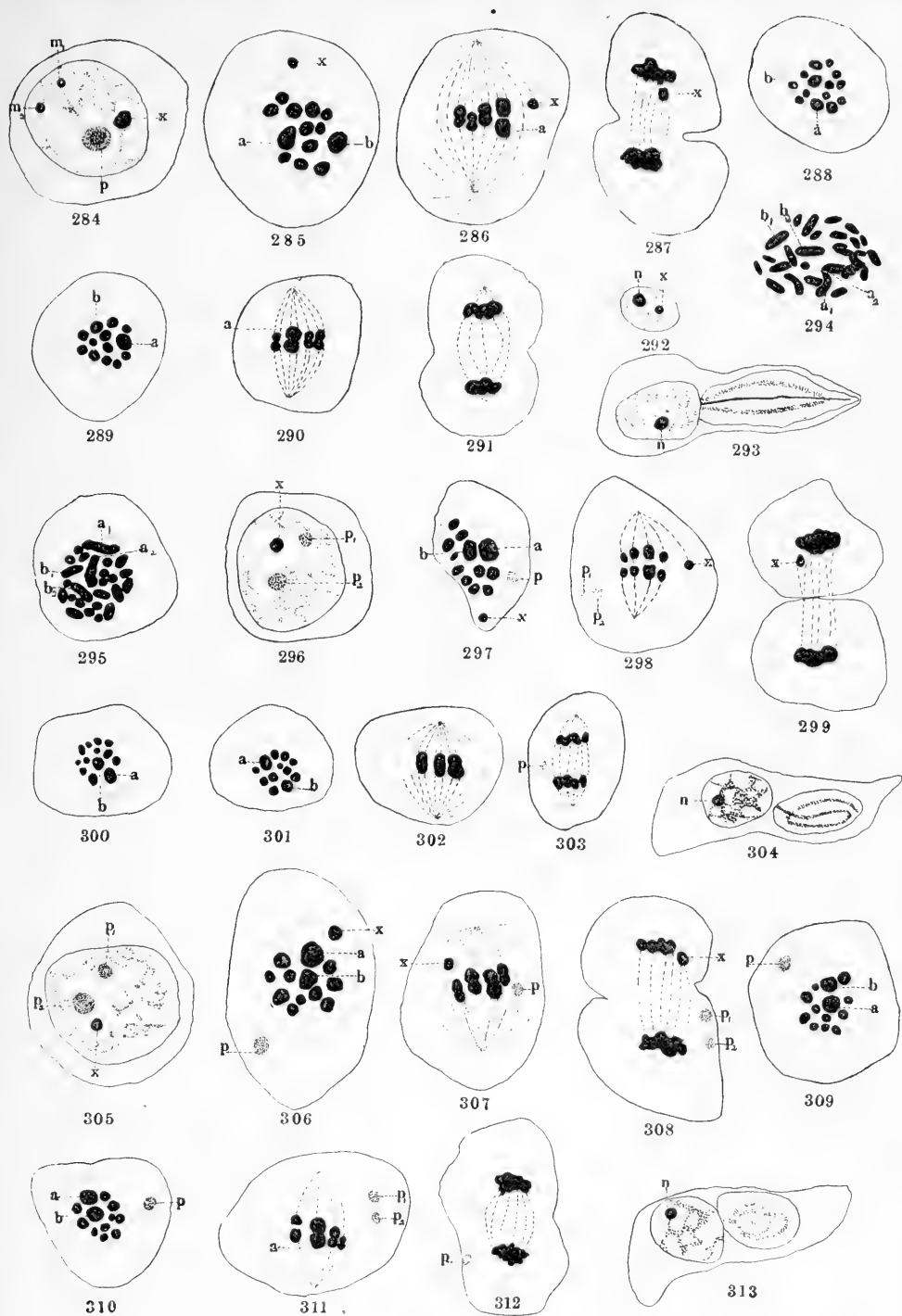
- Fig. 284 First spermatocyte, rest stage.  
Fig. 285 First spermatocyte, equatorial plate, 14 chromosomes.  
Fig. 286 First spermatocyte, metaphase.  
Fig. 287 First spermatocyte, anaphase.  
Figs. 288, 289 Second spermatocytes, equatorial plates, containing 14 and 13 chromosomes, respectively.  
Fig. 290 Second spermatocyte, metaphase.  
Fig. 291 Second spermatocyte, anaphase.  
Figs. 292, 293 Spermatids, half with  $x$ , half without.  
Fig. 294 Female somatic equatorial plate, 28 chromosomes.

*Amphiscepa bivittata* (Family Fulgoridæ)

- Fig. 295 Spermatogonial equatorial plate, 25 chromosomes.  
Fig. 296 First spermatocyte, rest stage.  
Fig. 297 First spermatocyte, equatorial plate, 13 chromosomes.  
Fig. 298 First spermatocyte, metaphase.  
Fig. 299 First spermatocyte, anaphase.  
Figs. 300, 301 Second spermatocytes, equatorial plates, containing 13 and 12 chromosomes, respectively.  
Fig. 302 Second spermatocyte, metaphase.  
Fig. 303 Second spermatocyte, anaphase.  
Fig. 304 Spermatid.

*Paciloptera bivittata* (Family Fulgoridæ)

- Fig. 305 First spermatocyte, rest stage.  
Fig. 306 First spermatocyte, equatorial plate, 13 chromosomes.  
Fig. 307 First spermatocyte, metaphase.  
Fig. 308 First spermatocyte, anaphase.  
Figs. 309, 310 Second spermatocytes, equatorial plates, containing 13 and 12 chromosomes, respectively.  
Fig. 311 Second spermatocyte, metaphase.  
Fig. 312 Second spermatocyte, anaphase.  
Fig. 313 Spermatid.



FULGORIDÆ

A. M. B. del.



# THE REACTIONS OF THE POMACE FLY, *DROSOPHILA AMPELOPHILA* LOEW, TO ODOROUS SUBSTANCES

BY

WILLIAM MORTON BARROWS

WITH FIVE FIGURES

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## I INTRODUCTION

*Drosophila ampelophila* is a small fly about three millimeters in length belonging to the family *Drosophilidæ*. It lays its eggs on fermenting fruit, which serves as food for both the larvæ and the adults. The ease with which large numbers of these insects can be reared in the laboratory during the winter as well as the summer, and the definiteness with which they react to many forms of stimuli, make them favorable subjects for experimentation. Since they find their food with great certainty even in the dark, a habit that seemed to involve the sense of smell, I was led to take up an investigation of their reactions to odorous substances.

Where the flies were abundant, it was noticed that they often entered bottles and other receptacles containing alcohol. The fact that the fermenting fruit upon which they feed is continually generating alcohols and other related compounds, led me to suspect that it was these substances that served to attract the flies, and that they therefore probably presented a clear case of chemotropism among air-inhabiting animals.

The experiments recorded in this paper were undertaken to determine, if possible, first, to what substances *Drosophila* is chemotropic, and secondly, in what way the fly finds its food.

The work was carried on in the Zoölogical Laboratory of Harvard University, under the direction of Prof. W. E. Castle and Prof. G. H. Parker, to whom I am indebted for much valuable advice and careful criticism.

## II EXPERIMENTS

### I *Preliminary Experiments*

For the preliminary experiments, which were planned to ascertain whether certain substances were stimulating or not for the flies, the following apparatus was devised. A two-dram vial closed at the end by a cork stopper was arranged as a trap. Piercing the stopper and reaching nearly to the bottom of the vial was a glass tube with a caliber of about two millimeters (Fig. 1). The bore of the tube was large enough to allow a fly to creep through easily and yet small enough to make it difficult for the fly to turn around after having once started into the tube. The tube projected beyond the cork on the exterior about three millimeters. If a fly once got halfway down the tube leading into the vial, the chance of its backing out or finding its way out later was very small. About 1 cc. of the substance to be tested was placed on a piece of filter paper in the vial. Five vials thus charged with substances to be tested usually formed the set of traps. At least one of these was always used as a control in that it contained filter paper wet with distilled water only or with some other material used as a check.

The traps, with their open ends directed toward the light, were placed in a vertical glass cylinder 20.5 cm. high and 17.5 cm. in diameter. The bottom edge of the cylinder rested on a sheet of clean filter paper and the top was closed by a glass plate. The atmosphere in the cylinder was kept moist by the evaporation of distilled water exposed in a small vessel. Many hungry flies, usually one hundred, were liberated in the glass cylinder and left there for twenty-four hours. By hungry flies is meant those which

had been supplied with distilled water but had been kept from food for twenty-four hours. If they are kept without food much longer than this, they begin to die and few survive sixty hours. After the flies had been allowed twenty-four hours in which to enter the traps, the experiment was discontinued and the individuals in each trap were counted. In this way some idea of the influence of different substances on the movements of the flies could be ascertained.

These experiments were preliminary, in that they aimed only to determine what substances called forth positive reactions. The

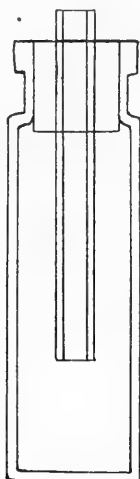


Fig. 1 Vial arranged as a trap.

results, which are necessarily fragmentary, because of the difficulty of dealing with odors in a quantitative way, are given in Table I.

From Table I it is apparent that of the ten substances which were tested singly in aqueous solutions, the flies gave definite positive reactions to four, namely amyl, and especially ethyl alcohol, acetic and lactic acid (Experiments 1, 2, 3, 5, 6). The remaining substances to which the flies did not react positively or did not react in numbers large enough to be significant, are propyl alcohol, butyric acid, valerianic acid, glucose, amyl valerianate and acetic ether. Experiments which were undertaken subse-

TABLE I

*Numbers of flies out of one hundred, which, in each of eight experiments, entered the several traps charged with odorous substances. The duration of each experiment was twenty-four hours*

No. of Experiment	Substance in the trap	No. of flies in trap
1.....	1 Acetic acid 4 per cent.....	0
	2 Alcohol 10 per cent.....	4
	3 Water (control).....	1
	4 Acetic acid 4 per cent with trace of acetic ether.....	5
	5 Glucose and water.....	0
2.....	1 Acetic acid 4 per cent.....	1
	2 Alcohol 10 per cent.....	6
	3 Water (control).....	0
	4 Acetic acid 4 per cent with trace of acetic ether.....	61
	5 Glucose and water.....	0
3.....	1 Alcohol 10 per cent containing acetic ether .04 per cent.....	85
	2 Alcohol 10 per cent.....	12
	3 Water (control).....	0
	4 Acetic acid 4 per cent.....	2
	5 Acetic acid 4 per cent containing acetic ether .04 per cent.....	19
4.....	1 Alcohol 10 per cent with trace of butyric acid.....	43
	2 Alcohol 10 per cent containing 2 per cent hydrochloric acid.....	0
	3 Alcohol 10 per cent (control).....	26
	4 Alcohol 10 per cent with trace of valerianic acid.....	14
	5 Alcohol 10 per cent with trace of isoamyl acetate.....	0
5.....	1 Water with trace of valerianic acid.....	0
	2 Butyric acid 2 per cent.....	1
	3 Water (control).....	2
	4 Propyl alcohol 2 per cent.....	0
	5 Water with a trace of amyl valerianate.....	0
6.....	1 Water with a trace of acetic ether.....	0
	2 Lactic acid 2 per cent.....	6
	3 Water (control).....	0
	4 Amyl alcohol 2 per cent.....	3
7.....	1 Alcohol 10 per cent with a trace of acetacetic ester.....	0
	2 Alcohol 10 per cent with a trace of isobutyl acetate.....	9
	3 Alcohol 10 per cent (control).....	5
	4 Alcohol 10 per cent with a trace of methyl acetate.....	29
	5 Alcohol 10 per cent with a trace of isoamyl acetate.....	8
8.....	1 Alcohol 10 per cent containing .05 per cent osmic acid.....	2
	2 Alcohol 10 per cent containing 2 per cent hydrochloric acid.....	0
	3 Alcohol 10 per cent (control).....	1
	4 Alcohol 10 per cent containing 2 per cent nitric acid.....	0
	5 Alcohol 10 per cent containing 2 per cent acetic acid.....	8

quent to those under discussion, however, showed that the flies did enter or attempted to enter traps which contained acetic ether in aqueous solution, so that it is possible that under other conditions the flies might be positive to some of the substances to which in these preliminary experiments they seemed not to be.

In Experiment 2 one trap was charged with 4 per cent acetic acid and another trap with 4 per cent acetic acid containing a small amount of acetic ether. The number of flies found in the trap which contained the mixture of acid and ether greatly exceeded that in the trap charged with acid only. A similar contrast was observed in Experiment 3. This increase of flies in the trap containing the mixture is evidently due to the acetic ether. A similar phenomenon was seen when to 10 per cent ethyl alcohol, a small amount of acetic ether, isobutyl acetate, or methyl acetate was added (Experiments 3 and 7). Isoamyl acetate may possibly also be classed with these substances, though the results of Experiments 4 and 7 do not show an entire agreement in this respect. An increase was also obtained when acetic or butyric acids were added in small amounts to the alcohol. This property may be slightly shared by valerianic acid and possibly by osmic acid, but it is certainly not a characteristic of hydrochloric and nitric acids which seem to be strongly repellent (Experiments 4 and 8).

All the organic substances tested in these preliminary experiments are found in fermenting fruits and the test conditions which gave the highest positive numerical results are probably those which simulated most closely the natural optimum conditions.

## 2 *Experiments with Alcohol, Acetic Acid and Acetic Ether*

As a more complete analysis of the effect of some of the odorous substances used in the preliminary experiments was desirable, it was decided to test more fully acetic acid, ethyl alcohol and acetic ether. These were chosen because they are commonly found in fermenting fruit, the first two in quite large quantities and the third in traces. To make the tests more accurate, a new piece of apparatus was constructed in which two traps were so placed in the sides of a leaden trough that each fly as it passed



through the trough had a chance to enter the charged trap or the check trap or to react to the odor issuing from the former.

The plan and elevation of the apparatus used are shown in Figs. 2 and 3, respectively. On a wooden base *A*, some 30 cm. in length

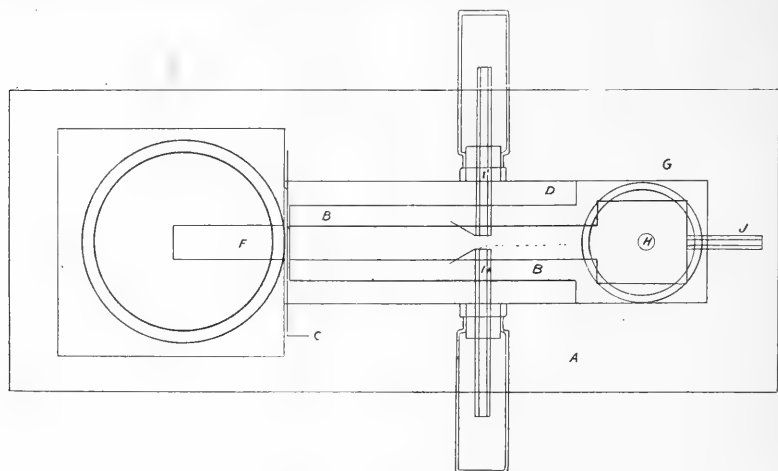


Fig. 2

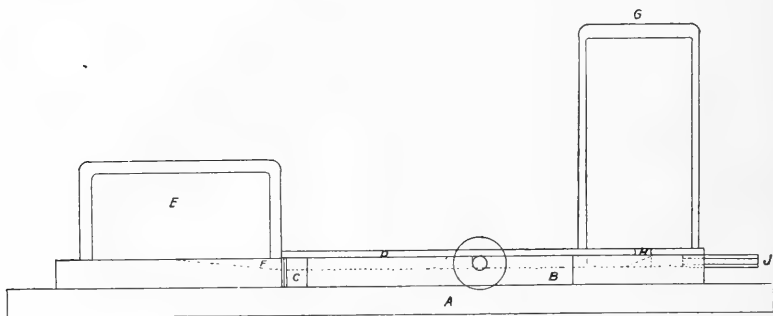


Fig. 3

Figs. 2 and 3 Plan and elevation of apparatus used in testing the reactions of *Drosophila* to odors. *A*, wooden base; *B*, leaden trough; *C*, zinc slide for closing exit from the receiving chamber; *E*, *D*, glass plate; *E*, inverted glass dish forming a receiving chamber; *F*, inclined way; *G*, inverted glass dish forming a collecting chamber; *H*, hole in glass plate serving as entrance to collecting chamber; *I P*, entrance tubes to trans; *J*, suction tube.

and 12 cm. in width, was mounted a leaden trough *B*, which was 14 cm. long and about 2.5 cm. broad except at the far end (right in the figure) where it expanded into a chamber 3 cm. square. The

passage in the trough was 5 mm. deep, and 11 mm. broad and extended from the near end, which was closed by a zinc slide *C* to the square chamber at the far end. The trough was covered by a glass plate *D*, which fitted to the lead closely enough to make it practically air-tight. Against the near end of the trough was an inverted cylindrical glass dish *E*, which served to hold the flies to be tested. This dish was raised to the level of the glass plate by a block of wood. Its chamber communicated with the passage of the trough by a short inclined way *F*, which allowed the flies to pass into the trough when the slide *C* was open. At the opposite end of the trough and resting upon the glass plate was another inverted glass vessel *G*, communicating with the trough by means of a small hole *H* through the glass plate. This vessel served as a reservoir to hold the flies that had been tested.

A fly creeping from chamber *E* along the trough to chamber *G* passes between the open ends of the two traps, which were inserted opposite each other through the walls of the trough at *I* and *I'*. A small glass tube piercing the far wall of the chamber at *J* was connected by a rubber tube with a suction apparatus, by means of which a current of air could be drawn through the trough at any desired rate. The suction apparatus consisted of a large bottle filled with water, closed at the top by a stopper with two holes. Through one hole was inserted a bent glass tube, which served as a siphon. The other hole was filled by a short glass tube which connected with the rubber tube from *J* and served to admit the air under external pressure to the partial vacuum formed by the siphon. Reference to Figs. 2 and 3 will show that when the siphon was allowed to run at a given rate, controlled by a clamp on the rubber tube, a current of air flowed from *E* through the trough, past the ends of the traps to the outlet *J*. The aim was to have this air current carry all the escaping odorous particles away from the mouth of the trap. To test the apparatus, hydrochloric acid was allowed to evaporate in chamber *E* and this gas was drawn by the air current along the trough and past one of the traps which was charged with ammonia water. White fumes of ammonium chloride were formed at the mouth of the trap *I*, and deposited along the path of the current. The dotted line in Fig. 2

marks the edge of the current of ammonium chloride, which shows that none of the ammonia moved against the current toward *E*.

In using the apparatus it was so placed that the flies, which are positively phototropic, would creep under the influence of light from chamber *E* to chamber *G*. A number of hungry flies were placed in chamber *E*; the current of air was then started; and the two vials were placed in position, one containing the substance to

TABLE II

*The numbers of flies which reacted positively to each of eight different strengths of ethyl alcohol. In each experiment the number of flies used was one hundred*

Number of the experiment	Strengths of alcohol in per cent	Number of flies that entered the		Number of flies that turned toward but did not enter the		Total number of reactions to alcohol in per cent	Total number of reactions to control in per cent
		Alcohol trap	Control trap	Control trap	Alcohol trap		
1.....	100	0	0	0	0	0	0
2.....	75	0	1	0	1	0	2
3.....	50	0	3	10	0	10	3
4.....	25	2	0	15	1	14	1.5
5.....	25	3	2	8	0		
6.....	20	7	0	9	0	16	0
7.....	15	2	1	6	0	11	1
8.....	15	2	0	12	1		
9.....	10	0	1	1	0	7.5	1
10.....	10	10	1	4	0		
11.....	5	0	3	0	1	3	1.75
12.....	5	2	0	3	0		
13.....	5	1	1	1	2		
14.....	5	0	0	5	0		
15.....	water	0	1	2	2	2	3

be tested, the other containing water used as a control. The slide was then opened far enough to allow a few flies at a time to pass down the trough toward the light. The flies, that reacted positively by turning abruptly toward the traps, were counted as were those that entered either of the traps. After fifty flies had been admitted to the trough, the slide was closed and the traps were interchanged. Now fifty more flies were allowed to pass through

the apparatus and their reactions recorded. These records added to those of the preceding fifty constituted the records of one experiment. When the different strengths of the same substance were tested on different days, the last strength used on the previous day was first tested in order to be sure that the hungry flies in stock had remained uniform in their response to this stimulus. Having ascertained this, the experiments were carried forward as though they formed a continuous series. The results of these experiments are given in Tables II to VI.

TABLE III

*The numbers of flies which reacted positively to glacial acetic acid and to different strengths of this acid in water. In each experiment the number of flies used was one hundred*

Number of the experiment	Strengths of acetic acid in per cent	Number of flies that entered the		Number of flies that turned toward but did not enter the		Total number of reactions to acid in per cent	Total number of reactions to control in per cent
		Acid trap	Control trap	Control trap	Acid trap		
1.....	glacial	0	0	5	1	5	1
2.....	50	0	0	6	0	6	0
3.....	25	0	0	12	7	11	4
4.....	25	0	0	10	1		
5.....	20	0	0	7	0	7	0
6.....	15	1	1	12	0	13	1
7.....	10	1	0	25	0	26	0
8.....	5	18	1	16	0	34	1
9.....	4	3	0	9	0	12	0
10.....	2	1	0	2	0	3	0

From Table II it can be seen that the greatest number of positive reactions to alcohol were obtained at 20 per cent concentration, while strengths above or below this grade show a decrease in the number of positive reactions.

It will be seen from Fig. 2, that a fly in passing from the near end to the far end of the trough enters the area of stimulation obliquely. Consequently one side of the animal must be stimulated before the other. Many of the flies entering the odor in this way give a positive reaction by turning toward the stimulated side.

This reaction indicates that the flies respond to a difference in the intensity of the stimulus on the two sides of their bodies.

The following peculiar response was very often observed. After flies had passed the traps they would often suddenly turn and run back with a characteristic zigzag motion to the mouth of the charged trap. While testing with 50 per cent alcohol, it was noted that about one-third of the flies reacted in this manner. Evidently they are able to follow the current of odor back to its source. These responses will be further discussed in a subsequent part of this paper.

TABLE IV

*The numbers of flies which reacted positively to each of three different strengths of acetic ether in water. In each experiment the number of flies used was one hundred*

Number of the experiment	Strengths of acetic ether in per cent	Number of flies that entered the		Number of flies that turned toward but did not enter the		Total number of reactions to ether in per cent	Total number of reactions to control in per cent
		Ether trap	Control trap	Ether trap	Control trap		
1.....	8	0	7	33	6	19	7
2.....	8	0	1	5	0		
3.....	4	0	0	9	0	8	1
4.....	4	0	0	7	2		
5.....	2	0	0	13	0	11.5	1
6.....	2	0	0	10	2		

It is plain from an inspection of Table III that the largest number of positive reactions was produced by 5 per cent acetic acid. Not only is this true, but, when the trap was charged with this strength, about half of the flies which at first failed to respond to the stimulus returned through the current of odorous material from the far end of the trough back to the mouth of the trap. These responses are not included in the table.

Acetic ether is soluble in water only to the extent of about 8 per cent, and when used in such high concentration it affects the flies in a singular manner. They show intense excitement and struggle at the mouth of the trap for a chance to enter, yet when one has succeeded in entering it backs out almost immediately.

It is probable that the dissolved ether evaporates rapidly forming an almost saturated atmosphere inside the trap, and this is known to kill the flies in less than three minutes. Hence the excessive stimulation probably causes them to back out of the entrance to the trap into which they had been enticed by the less concentrated vapor. Acetic ether is never so abundant in decaying fruit as in the weakest solutions (2 per cent) tested in these experiments.

In order to make a mixture which should combine the optimum strengths of alcohol and acetic acid, equal volumes of 40 per cent alcohol and 10 per cent acetic acid were mixed. This mixture then contained 20 per cent of alcohol and 5 per cent of acetic

TABLE V

*The numbers of flies which reacted positively to each of four different strengths of a mixture of equal parts of 40 per cent alcohol and 10 per cent acetic acid diluted with water. In each experiment the number of flies used was one hundred*

Number of the experiment	Strengths of the mixture in per cent	Number of flies that entered the		Number of flies that turned toward but did not enter the		Total number of reactions to mixture in per cent	Total number of reactions to control in per cent
		Charged trap	Control trap	Charged trap	Control trap		
1.....	100	13	0	17	4	30	4
2.....	50	26	5	2	0	28	5
3.....	25	29	1	2	0	31	1
4.....	12.5	27	5	12	0	39	5

acid. Table V shows the results obtained by testing flies with this mixture either pure or diluted with water.

A solution of 12½ per cent of this mixture, which is equal to a mixture of 2½ per cent alcohol and ⅝ per cent acetic acid, gives a slightly higher number of positive reactions than is given either by 5 per cent acetic acid (Table III) or 20 per cent alcohol (Table II). The numbers of the positive reactions are not significantly large, yet it is probable that the mixture is uniformly more stimulating than alcohol or acetic acid alone.

Table VI shows the results obtained by testing the mixture containing 20 per cent alcohol and 5 per cent acetic acid (Table V) to

which had been added 8 per cent of acetic ether. Of the dilutions used,  $12\frac{1}{2}$  per cent of the mixture induced the largest number of flies to react positively. It is probable that the experiments were complicated by the presence of a higher per cent of acetic ether than is met with under natural conditions. In Table I, Experiment 3, about .04 per cent of acetic ether was added, respectively, to 10 per cent alcohol and to 4 per cent acetic acid, and in both instances there was an immense increase in the number of responses as compared with the responses to those reagents alone; this increase must have been due to the slight amount of acetic ether present. We may safely conclude that acetic ether probably plays some part in the reactions of *Drosophila* to normal food.

TABLE VI

*The numbers of flies which reacted positively to each of three different strengths of the mixture of alcohol, acetic acid and acetic ether. In each experiment the number of flies used was one hundred*

Number of the experiment	Strengths of the mixture in per cent	Number of flies that entered the		Number of flies that turned toward but did not enter the		Total number of reactions to mixture in per cent	Total number of reactions to control in per cent
		Charged trap	Control trap	Charged trap	Control trap		
1.....	100	0	1	4	0	4	1
2.....	$12\frac{1}{2}$	13	3	7	0	20	3
3.....	$6\frac{1}{2}$	4	0	5	0	9	0

The foregoing experiments show that *Drosophila* is positively chemotropic to alcohol, acetic acid and under certain conditions to acetic ether. The optimum strengths of alcohol and of acetic acid are 20 and 5 per cent, respectively, while that of acetic ether is uncertain, but must be only a fraction of 1 per cent.

Table VII, made up from data given by Leach ('05), shows that alcohol and acetic acid are commonly found in cider vinegar, fermented cider, and California sherry in per cents that are close to those which call forth the largest numbers of reactions in *Drosophila*.

Acetic ether is found in these fluids in very slight traces.

## 3 Experiments on the Directive Effects of Odorous Substances

Having determined that these flies are chemotropic to fermenting fruit, I turn to the second question, In what way does the fly find its food?

To ascertain the accuracy of flight toward the food, experiments were carried out in the following way. About one hundred hungry flies were liberated in a large laboratory room. A few minutes after their liberation a tumbler, freely exposed on the top of a table and containing fermenting banana was opened. As the hungry insects in flying through the room passed near the table they eventually discovered the banana. When they were six or more feet from the tumbler they showed a rather characteristic

TABLE VII

*Amounts (in per cent) of acetic acid, and alcohol found in cider vinegar, fermented cider, and California Sherry (Leach '05)*

Substances	Acetic acid in per cent			Alcohol in per cent			Number of samples
	Max.	min.	Av.	Max.	Min.	Av.	
Cider vinegar....	7.61	3.24	4.65				44
Fermented cider.	6.59	.24	3.18	6.85	1.1	4.72	16
California sherry	.79	.25		21.85	8.22		66

vibratory flight. Short rapid excursions were made through the air, up and down, forward and backward, right and left. Sometimes the fly came nearer the tumbler and under such circumstances it often remained in its vibratory flight in this new situation. As it approached to within about three feet of the tumbler the excursions shortened and the fly oriented more accurately to the source of the odor, though the flight still showed considerable vibration to right and left while the head of the fly was directed generally toward the tumbler. When about two feet from the tumbler, the vibratory movements grew less and less extensive and the flight became more rapid and more accurately directed toward the tumbler. The last six or eight inches of the journey was made in nearly a straight line to the edge of the tumbler or to its base.



It is a source of continual surprise to see how accurately and quickly many flies will find food. Not only do the flies find food easily and certainly during flight, but they can also find it successfully when creeping. To test this the following experiments were tried. A small piece of fermenting banana was placed on a glass plate one inch square and the glass plate with the banana was put in the center of a square sheet of paper ruled into 25 squares each one an inch on a side (Fig. 4, *a* to *b*). The glass cylinder used in the first experiments (p. 516) was then placed over this paper in such a position that the four corners of the paper just touched the lower edge of the cylinder. To prevent air currents from driving the odorous particles away, the chamber was closed by a glass plate above. Hungry flies were admitted singly at the bottom of one side of the chamber. They flew as usual toward the light side and upward, but in the course of a minute started to creep down the glass toward the bottom of the chamber. As they came on the paper their course was carefully plotted on a duplicate sheet of ruled paper. The courses given in the diagrams (Fig. 4) show the characteristic paths traversed by twelve flies.

These paths show that in most cases the flies took the most direct route in order to reach the food, *i.e.*, they did not merely run upon it by chance. The paths are usually so direct that it appears as if the flies found the food by sight, but that this is not so, is shown by subsequent experiments, in which after the removal of the antennæ the flies seldom found the food, though their eyes were intact.

It is clear that both in flight and in creeping the movements toward food are at first irregular and afterward more accurately directed, so that the fly eventually takes an almost straight course to the food. The beginnings of the courses in flight and in creeping are such as to suggest the trial and error method of response. This view is supported by what is sometimes seen in the zigzag course taken in the return of flies against the current of odor in the trough in the experiment described on p. 524. The conclusions of the courses however in both flight and creeping are so accurately directed toward the food that trial and error can play no part in explaining this condition; one is forced on the contrary to assume

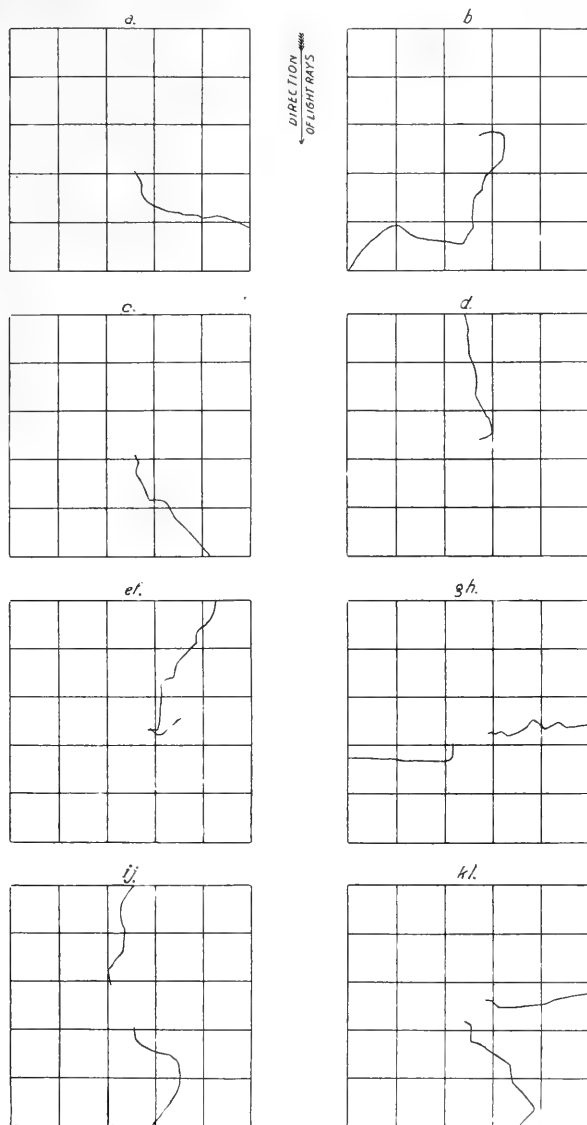


Fig. 4 The irregular lines represent the paths traversed by twelve flies in reaching a piece of fermenting banana placed in the center of the area. The area was 5 inches square and the fly almost always started from some point on the outer edge of the area.

some such method of orientation as is implied in the theory of tropisms. The more usual conception of the tropism theory, as advocated particularly by Verworn, is to the effect that when an animal is unsymmetrically stimulated it turns until it is symmetrically stimulated and either faces toward or away from the source of the stimulus and then moves in the appropriate direction. It is evident from this that symmetrical stimulation is an essential feature of this theory. Loeb has extended this view in the sense that he often implies that the stimulus acts directly on the locomotor organs, but I do not regard this as an essential part of the tropism theory, and, as I shall show presently, this modification of the theory has no application in this case. The question, then, is, are the accurately directed responses of *Drosophila* dependent on symmetrical stimulation? If this is the case, one would naturally turn to the antennæ of this animal as the symmetrical receptive organs of smell for such reactions.

#### 4 *Experiments to Determine the Position and Function of the Olfactory Sense Organs*

It is generally believed that in most insects the antennæ are the seat of the olfactory organs. In some species these organs are placed in pits, while in others they are exposed on the surface of the antenna. Mayer ('79) described a pit in the third segment of the antennæ of a species of *Drosophila* which he considered an olfactory organ. I have found that *Drosophila ampelophila* has a large sac-like pit situated in the end of the terminal (third) segment of the antenna, which contains sense cones. Fig. 5 shows a front view of the head of *Drosophila* and the position of this pit in the left antenna. The following experiments on flies which had been deprived of the third segments of their antennæ show that without doubt the olfactory organ in *Drosophila* is located in this segment.

It was found by repeated trials that the antennæ could not be satisfactorily covered with gum to keep out the stimulating odor, nor could they be burned off without considerable injury to the fly. The method finally employed was to place a fly, already etherized, on its back on a glass slide and to hold it down with a small camel's-

hair brush, which in turn was held in place by a small rubber band. Secured in this way under the lens of a high-power dissecting microscope, the third joints of the antennæ were cut off by a pair of fine embryological dissecting scissors. There is a deep division between the second and third segments of the antenna, and the third segment was usually removed without injury to the second. After the effect of the ether<sup>1</sup> had passed away, the fly operated upon seemed in most respects perfectly normal. Such flies were left for twenty-four hours without food, but were supplied with

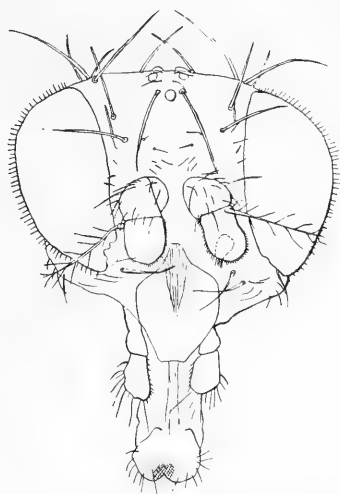


Fig. 5 Front view of the head of *Drosophila* showing in dotted lines the position of the olfactory pit in the terminal segment of the left antenna.

water. At the end of this time they were liberated singly in the large cylinder used in the experiments described on p. 516, and were carefully watched for five minutes in order to be certain that their behavior was normal. Having ascertained this, their ability to find a piece of fermenting banana in the cylinder was tested. The time required to find the food was recorded, or if the food was not found in fifteen minutes, the experiment was discontinued.

<sup>1</sup> It should be noted that the process of etherizing has no lasting effect on the ability of the flies to scent food, *i. e.*, normal flies after recovery from ether find food with as great certainty as they did before etherization.

Of fourteen flies which were thus tested all with one exception failed to find the food within fifteen minutes. In the exceptional case food was found in twelve minutes, but the insect's course was such that it obviously came to the food by accident. During these experiments the flies were carefully watched, and although many of them came within 1 cm. of the food, they did not find it.

To test this matter further, four of the flies with defective antennæ were allowed to wander in a small glass tumbler until they found the food, which they apparently did by accident. When one foot of the fly touched the food or a small drop of water the tongue was immediately put down and the animal began feeding. It is not impossible that two transparent hairs which are found beneath the claws of each front foot, and have the appearance of sense hairs, may be instrumental in giving rise to this feeding reaction. It therefore seems certain that the sense of smell is absent, or at least greatly reduced, in flies which have lost the terminal joints of both antennæ. In order to determine the relative time taken to find food by flies with and without the terminal segments of the antennæ, six normal flies were admitted to the chamber and the time recorded which elapsed before they reached the food. These flies were then operated upon; the distal segments of both antennæ were cut off and they were allowed to rest twenty-four hours, when they were again tested and the time similarly recorded. The results of these experiments are given in Table VIII.

From this table it will be seen that the normal hungry fly finds the food in about two and one-half minutes, while the same fly after having been operated upon seemed unable to locate the food.

We may conclude: first, that *Drosophila* does not find its food by sight, but by smell, and when this sense is lost it reaches the food only by accident; and, secondly, that the olfactory sense organs—at least those which are concerned with finding food—are localized in the third or distal segment of the antenna.

The fact that in *Drosophila* the antennæ are the principal organs concerned in the reception of olfactory stimuli and that they are symmetrically placed on the body of the animal, leads to the conclusion that these flies orient to odorous centers in the way

assumed by the tropism theory. Forward locomotion would be called forth by an equal stimulation of the two antennæ and lateral movements by an unequal stimulation of these organs. If this view is correct, circus movements ought to result after the removal of one antenna even though the stimulating atmosphere contains a uniformly distributed odorous substance. It was, therefore, thought desirable to experiment upon flies from which one antenna had been removed in order to produce excessive unilateral stimulation.

Before the operation the flies were tested for five minutes in pure air and five minutes in an atmosphere with odor, to make certain that they were normal, *i. e.*, that they did not turn more

TABLE VIII

*Records of the times which six flies took to find food before and after the terminal segments of their antennæ had been removed*

Numbers of the flies	Time in minutes in which the	
	Normal flies found the food	Injured flies found the food
1.....	2.5	Flies all failed to find the food at the end of twenty minutes.
2.....	4.0	
3.....	1.75	
4.....	3.5	
5.....	2.0	
6.....	1.0	
Average.....	2.45	

frequently to the right than they did to the left or vice versa. The terminal segment of the right or the left antenna was then cut off from each fly. After the removal of this segment, the flies were fed and allowed to remain twenty-four hours, when they were again hungry. They were then admitted singly to the cylinder containing only pure air and watched for five minutes. Without exception they reacted as they did under similar circumstances previous to the operation. A little odor was now blown into the cylinder from a wash bottle partially filled with fermenting banana and the flies were again admitted singly and their movements carefully watched. If the fly moved in a circular path, as was

usually the case, a record of one circle was put down for the fly when it had turned continuously through an arc of 360 degrees in one direction. A circle was called positive if the fly moved with its normal antenna on the side toward the center and negative if it moved in the opposite direction; *i. e.*, a fly having its left antenna cut and moving always with its right side next the center would be said to be describing a positive circle, etc. Table IX shows the records of twelve flies tested in the manner described.

TABLE IX

*The numbers of positive and negative circles made by twelve flies which had only one functional antenna. Each fly was tested singly for a period of five minutes in a uniformly odorous atmosphere*

Numbers of the flies	Normal antenna	The number of circles made in a	
		positive direction	negative direction
4.....	right	6	0
5.....	left	4	0
7.....	right	4	0
1.....	left	3	0
2.....	left	5	1
3.....	right	5	1
4a.....	right	5	0
6.....	left	2	0
7a.....	left	2	0
8.....	left	5	0
9.....	left	2	0
10.....	left	3	2
Totals .....		46	4

From this set of experiments it will be seen that forty-six out of fifty of the circles, or 92 per cent, were made in a positive direction, *i. e.*, toward the normal antenna. As this antenna is obviously the one stimulated, it is clear that the flies must orient to unequal, unilateral stimulation.<sup>2</sup>

<sup>2</sup> Since this paper was written Kellogg (1907, p. 153) has recorded circus movements in the males of the silkworm moth after the removal of one antenna and on exposure to odors.

## III THEORETIC DISCUSSION

The experimental results recorded in this paper show very conclusively that the reactions of *Drosophila* to odorous materials are by no means uniform, but vary in method under different circumstances. When the stimulus is very weak little more than random movements are excited, but when the stimulus is somewhat stronger trial and error movements gradually prevail, whereby the fly becomes approximately oriented toward the odorous material, much as has been emphasized for many lower animals by Jennings ('04). Finally the orientation to the odorous material becomes very accurate and the fly may be said to take an almost direct course to it. It is clear that the latter part of the course is accomplished by methods in the main free from anything that can be described as trial and error. Since under a like degree of stimulation flies, after the loss of an antenna, carry out circus movements with great regularity, it seems impossible to explain the movements under these conditions in any other way than on the basis of the tropism theory. This theory has been stated in several ways. As applied to chemical stimulation Verworm ('99, p. 429) declares: "The word chemotaxis is applied to that property of organisms that are endowed with the capacity of active movement by which when under the influence of chemical stimuli acting unilaterally they move toward or away from the source of the stimulus. Where there is an approach to the source of the stimulus, there is *positive* chemotaxis, where there is a removal from the source *negative* chemotaxis. Unilateral stimulation with chemical stimuli is only realized when the concentration of the substance in question gradually increases from the living object in one direction."

The method by which *Drosophila* finds its food is directly comparable to that observed by Harper ('05, p. 33) in the reactions of *Perichæta* to weak and strong light. This earthworm orients away from the source of a weak light stimulus by frequent random movements, *i. e.*, by the trial and error method. But when the light stimulus is greatly increased the orientation is direct, random movements toward the light are suppressed altogether and the



worm appears to move directly away from the light without noticeable trial movements.

It seems to me probable from experiments described by Pearl ('03, pp. 623-670) that planarians may follow some such method in finding food. However, as the animal is not highly specialized, the distance through which it can orient accurately is small and the result is not striking.

#### IV SUMMARY

1 *Drosophila ampelophila* is a small fly peculiar for its fondness for fermenting fruit.

2 These flies are positively chemotropic to amyl and especially ethyl alcohol, acetic and lactic acid and acetic ether.

3 Acetic ether, isobutyl acetate and methyl acetate, when added in small amounts to 10 per cent ethyl alcohol, greatly increase its attractiveness. A similar increase is noted where acetic or butyric acids are added to the alcohol. All these organic substances are found in fermenting fruits.

4 The optimum strengths of ethyl alcohol and acetic acid as determined by the number of positive reactions given to different strengths is 20 and 5 per cent, respectively, while a mixture containing  $2\frac{1}{2}$  per cent alcohol and  $\frac{5}{8}$  per cent acetic acid gives a slightly higher number of positive reactions than is given by either 5 per cent acetic acid or 20 per cent ethyl alcohol. Alcohol and acetic acid are commonly found in cider vinegar, fermented cider, and California sherry in per cents that are close to those which call forth the largest number of reactions in *Drosophila*.

5. *Drosophila* does not find its food by sight, but by smell, and when this sense is lost it reaches its food only by accident. The olfactory sense organs—at least those which are concerned with finding food—are located in the third or terminal segment of the antenna.

6 When one antenna is lost and the other antenna is stimulated by food odor, circus movements are carried out in such a way as to prove that the fly orients normally by an unequal stimulation of the antennæ.

7 *Drosophila*, when stimulated by weak food odor, first shows random movements, *i. e.*, it attempts to find the food by the method of trial and error, but as the fly passes into an area of greater stimulation, these movements give way to a direct orientation. This orientation is a well defined "tropism" response. These reactions of *Drosophila* are paralleled by those of *Perichæta* to strong and weak light and possibly also by the food reactions of planarians.

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## THE EFFECT OF TEMPERATURE ON THE MIGRATION OF THE RETINAL PIGMENT IN DECAPOD CRUSTACEANS

BY

EDGAR DAVIDSON CONGDON

WITH SEVEN FIGURES

### I INTRODUCTION

The last few decades have witnessed a gradual accumulation of knowledge concerning photomechanical changes in the retinal pigment of vertebrates, cephalopods and arthropods. It has also been shown that light influences the melanophores of the reptile skin much as it does the retinal pigment cells. The effects of temperature upon the pigment migration of the melanophores, especially in *Anolis* and *Phrynosoma*, have been discussed recently by Parker ('06). Only two reports of the effects of temperature on retinal pigment have appeared and these both refer to the frog. Kühne ('79, p. 334) stated that in frogs which were subjected to low temperature in darkness, the retinal pigment extended farther toward the light between the cones than it did in those which had been subjected to high temperature in the dark. Herzog ('05) subsequently investigated this subject at greater length. He agreed with Kühne that below 18° C. any decrease of temperature causes a distal migration of pigment and any increase, a proximal one. Above 18° C. he believed the result was the reverse.

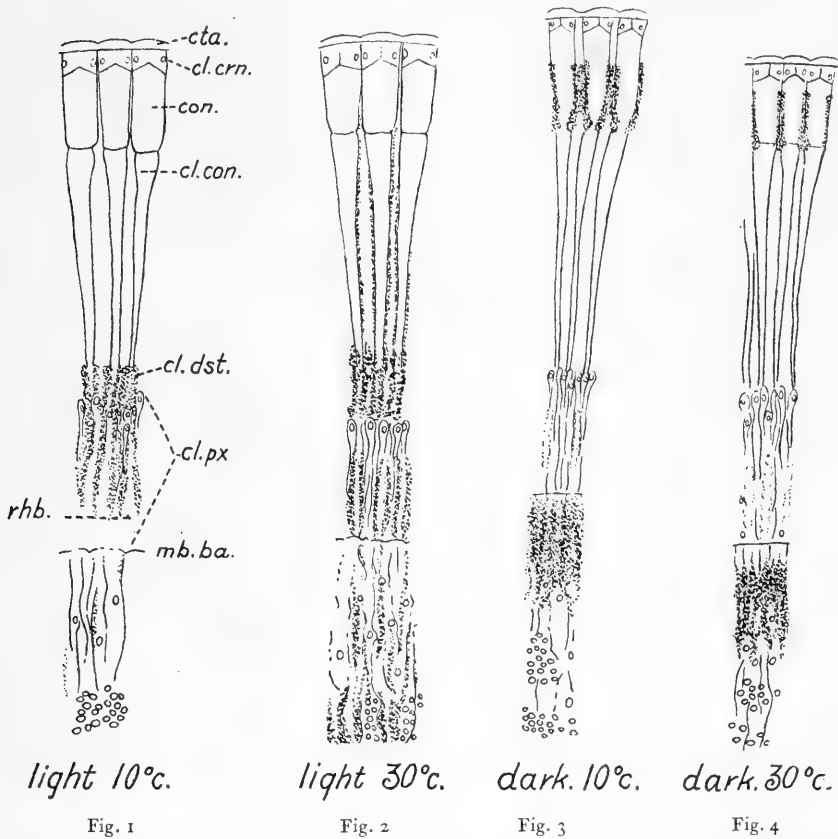
It was suggested to me by Prof. G. H. Parker that the decapod crustaceans would be favorable objects for the study of the influence of temperature upon pigment migration because of the marked photomechanical changes often found in their eyes. The prawn, *Palæmonetes vulgaris* Stimp., and the crayfish, *Cambarus bartonii* Gir., were chosen as being easily obtainable species whose photomechanical reactions were well known. The conditions found in *Palæmonetes* will be considered first.

## II PALAEMONETES VULGARIS STIMP.

The eyes of the prawn consist of a stalk and terminal bulb. The former contains a series of four optic ganglia, which are enlargements of the optic nerve. The bulb is made of numerous rod-like ommatidia, which extend somewhat radially from a basement membrane (Fig. 1, *mb. ba.*) near the end of the stalk to square facets (*cta.*) in the cuticula covering the bulb. An ommatidium may be roughly divided into a distal two-thirds, consisting chiefly of cone cells, and a proximal third containing the rhabdom. The four cone cells (*cl. con.*) lie parallel to the axis of the ommatidium, and in their distal portions are closely associated to form the glassy cone. They taper proximally from the cone to the rhabdom whence they possibly extend as processes to the basement membrane. Between the cone and the facet are two small corneal hypodermal cells (*cl. crn.*) Six distal reticular cells full of black pigment together form a sheath around the cone cells (Fig. 3). They do not belong exclusively to one ommatidium, but each one serves as a partial sheath for three cones. The lower third of the ommatidium contains seven proximal reticular cells (Fig. 1, *cl. px.*), lying close together and parallel to the ommatidial axis; they extend as long processes into the region proximal to the basement membrane. Distal to this membrane they unite to form the spindle shaped rhabdom (*rhb.*) They contain black pigment. Associated with them are one or two whitish accessory pigment cells. Fibrillæ from the distal optic ganglion extend up through the proximal reticular cells to end in the rhabdom. Distally, the central parts of the ommatidium are transparent and convey the light to the rhabdom, which is thus open to stimulation.

The photomechanical changes of *Palaemonetes* have been described by Parker ('97). In increasing light the distal reticular cells migrate as wholes in a proximal direction, thus restricting, as Exner ('91) has pointed out, the amount of light that enters the deeper parts of the eye. The pigment of the proximal reticular cells is at the same time carried distally along the sides of the rhabdom, probably by protoplasmic streaming within these cells. This process also reduces the amount of light that can reach the

rhabdom. In decreasing light the distal pigment cells move distally till they surround the cone and the proximal pigment is carried below the basement membrane. In consequence of the



Figs. 1 to 4 longitudinal sections of the ommatidia of *Palaemonetes*, magnified 200 diameters, showing the distribution of the retinal pigment under the following conditions:

Fig. 1, in light and at 10° C. *cl. con.*, cone cell; *cl. crn.*, corneal hypodermal cell; *cl. dst.*, distal reticular cell; *cl. px.*, proximal reticular cell; *con.*, cone; *mb. ba.*, basement membrane; *cta.*, cuticle; *rhb.*, rhabdom.

Fig. 2, in light and at 30° C.

Fig. 3, in dark and at 10° C.

Fig. 4, in dark and at 30° C.

movement of the proximal pigment, the light-colored accessory pigment about the rhabdom is exposed and may serve, as Exner

observed, for a reflecting apparatus. These pigment migrations plainly tend to protect the eye from over-stimulation by strong light and to increase its chances of stimulation in weak light.

It is evident from the foregoing that light conditions must be taken into account in testing the effects of temperature. To do this, a series of experiments in the dark and another in the light were planned, each of which included three temperatures: 10°, 20° and 30° C. One extreme of temperature in each series would increase and the other decrease the effect of light, if indeed temperature is a factor in the migration of pigment. As the photo-mechanical changes in *Palæmonetes* are ordinarily completed in about two hours, I extended my experiments to only two and a half hours. The three experiments of each series were performed at the same time. Care was taken in the light series to have the three aquaria for the three experiments placed close together so as to receive equal illumination. Six to twelve individuals were taken for each experiment. All of the animals came from a common supply and were similar in size and sex. One set of experiments was conducted twice, once with each sex. No difference in responsiveness was found between males and females.

The animals were put into quart glass jars filled with water and these jars were placed each in a two-gallon cylindrical aquarium. The latter was filled with water at the desired temperature; thus the water in the inner jar containing the animals was brought in half an hour to the required temperature. Preliminary trials showed that 10° C. and 33° to 35° C. were not harmful to the prawns when thus gradually produced, though the higher temperature would cause at times the death of the animals if suddenly applied. In the dark series there was no easy means at hand for maintaining the desired temperature in the dark-proof box without admitting light. Consequently the box was kept closed during the whole of the experiment, the water thus being allowed to change gradually toward room temperature. This resulted in a variation of about 3° C. during the experiment, an amount not sufficient to predjudice the result. At the end of treatment the animals were plunged into water at 80° C. for a fraction of a minute, until fixation was accomplished. The eyes were then prepared

for examination by being hardened, cut into sections and stained with borax carmine.

Eyes of the different experiments showed a very perfect series of migration stages both for the proximal and the distal pigment. In the dark series, the *proximal* pigment was always proximal to the basement membrane. At 10° C. (Fig. 3) it was close against the basement membrane; at 20° C. the distance of its distal margin below this membrane was equal to about one-fifth the length of the rhabdom; at 30° C. (Fig. 4) the distance was two-fifths of the length of the rhabdom. In the light series at 10° C. (Fig. 1) there was a strong concentration of the proximal pigment into the ends of the cells just distal to the rhabdom. Only a little pigment could be seen proximal to this. At 20° C. more of the pigment was proximal in position and surrounded the rhabdom. At 30° C. (Fig. 2) the pigment was rather dense around the rhabdom yet not so abundant as at the distal ends of the cells. Both series show that with increasing temperature the proximal pigment moves proximally and with decreasing temperature it moves distally.

The *distal* reticular cells can not be said to show as pronounced a response as the proximal ones did, yet the series was convincing. In all preparations from the light series the distal reticular cells were proximal to the cone; in the dark series they were at least partly surrounding it. At 20° C. in the light the distal pigment was in large part distributed evenly along the cone cells; a small part was collected at the top of the proximal reticular cells. Low temperature increased the effect of the light by massing all of the pigment just above the proximal reticular cells. The high temperature produced an even distribution of the pigment along the cone cells with no proximal accumulation. In the dark condition the distal pigment cells completely covered the cone at high temperature (Fig. 4). Low temperature (Fig. 3) resulted in a proximal migration equal to one-third the length of the cone. In general, increased temperature causes distal, and decreased temperature, proximal migration in distal reticular cells.

It may be said that in both types of pigment cells in *Palæmonetes* the effect of increased temperature is opposite to that of increased light.



Parker found a migration of the accessory pigment, but as it is not easy to determine this even in its relation to the light, I did not occupy myself with its relation to heat.

### III CAMBARUS BARTONII GIR.

In the crayfish *Cambarus bartonii* the reaction of the retinal pigment to changes in light and in temperature were by no means so clear as in the prawn. Exner ('91, pp. 108-109) long ago made a similar statement about *Astacus*, so far as light reactions were concerned.

In experimenting on *Cambarus* the factor of light was eliminated by confining the temperature treatment to animals in the dark. The series of temperatures tested was 2°, 10°, 14°, 22°, 29°, 34°, 39° and 41° C. The high temperatures 39° and 41° C. resulted in the death of half the animals and produced variable conditions in the eyes of the survivors. The experiments were discarded as probably dependent upon irregular moribund conditions. There was no reason to think that 2° C. was harmful. As a precaution against shock the more extreme temperatures were only gradually applied. Animals that were subjected to 2° C. were kept at this temperature for twelve hours, so that, if the cell processes were somewhat retarded by the cold, sufficient time would be given for the reaction to become complete. The animals were kept at the other temperatures for at least two and a half hours, as in the case of *Palæmonetes*. The low temperatures were obtained by putting the animals in a shallow dish supplied by water cooled by ice. For the high temperatures, a tank was arranged in a light-proof box so that water of a desired temperature could be replenished without admitting light. After the experiments the eyes of the animals were fixed in water at 80° C. The tough cuticula was then removed and the retina sectioned and stained in borax carmine.

The distal reticular cells of *Cambarus* showed in the different experiments a considerable diversity of positions and could not be reduced to a simple series conformable to the differences of temperature. I am inclined to ascribe this condition to some fault in

my methods. The average positions of the proximal pigment of different animals formed a natural series, although the average

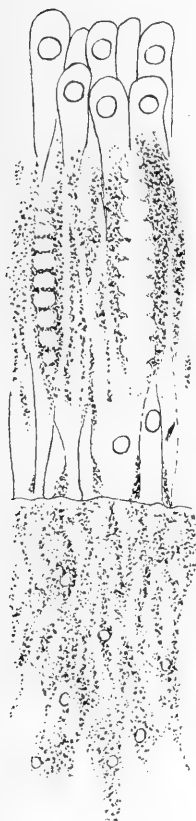


Fig. 5

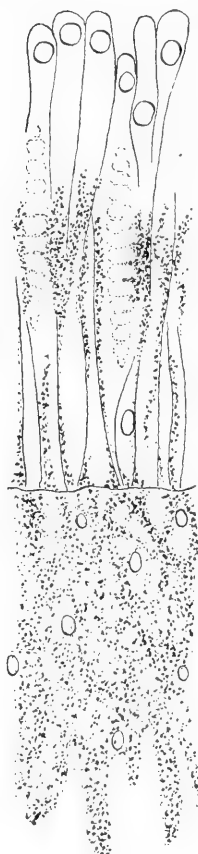


Fig. 6

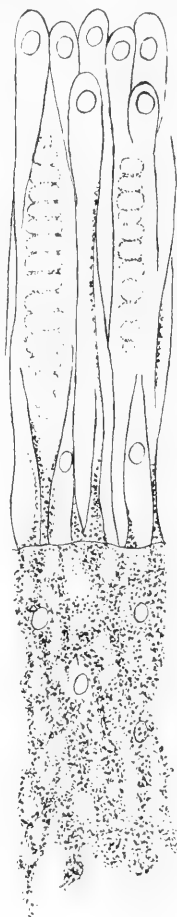


Fig. 7

Figs. 5 to 7 longitudinal sections of retinal and sub-retinal portion of the eye of *Cambarus*, magnified 350 diameters, showing the distribution of the retinal pigment under the following conditions:

Fig. 5, in dark and at 2° C.

Fig. 6, in dark and at 22° C.

Fig. 7, in dark and at 34° C.

interval between the temperatures employed was only about 6° C. At 2° C. (Fig. 5) a considerable amount of the proximal pigment

was found clustered around the rhabdoms or between them. Sometimes a little was scattered in the region between the rhabdoms and the basement membrane. Eyes of animals that had been kept at 34° C. (Fig. 7) frequently showed no pigment at all above the basement membrane. In some cases a small amount of pigment was scattered near the rhabdoms. Although there were frequent individual differences, the remaining experiments yielded a natural series of results between the two extremes mentioned. The proximal pigment of *Cambarus* moves therefore, like that in *Palæmonetes*, distally with decreasing temperature and proximally with increasing temperature. Light and heat have opposite effects. As in *Palæmonetes*, the movement in response to temperature is much less than in response to light.

#### IV DISCUSSION

Parker ('06, p. 410) in a recent paper summarized the effects of light on the melanophores of the reptile skin and on the proximal reticular pigment in the arthropods in the statement that all migration due to increased light is distal and so toward the source of light, and all migration due to decreased light is proximal. He also gives experimental evidence that increased temperature produces a proximal migration and decreased temperature a distal one in the melanophore pigment of reptiles. The proximal pigment of the decapod crustaceans *Palæmonetes* and *Cambarus*, as shown in this paper, falls under the same rule. Herzog's observations on the influence of temperature on the migration of the pigment in the frog's retina agrees with this statement for temperatures below 18° C., but not for those above this point, where the reverse is said to be true. Aside from this observation of Herzog's, which needs confirmation, all evidence points toward a general law for temperature the reverse of that for light; namely, increase of temperature causes proximal migration, decrease of temperature, distal migration.

In most instances of the migration of retinal pigment, the process has an adaptive value in controlling the amount of light that reaches the receptive organs. Possible adaptations may also be

easily pointed out in the migration of the melanophore pigment of reptiles. On the other hand, the migration of the retinal pigment in crustaceans as caused by change of temperature seems to me not to be adaptive, for it is always small in amount and it occurs at temperatures higher and lower than those commonly experienced by the animals. It seems reasonable that the migration is closely associated with the accelerating effect of heat on the chemical changes in the melanophore cell, and even if the migration were more marked, it is difficult to see what advantage it would give to its possessor. A probably related instance of lack of adaptation has been described by Hess ('05, p. 423) in the cephalopod eye, which often required one to two days in which to complete the pigment migration. This length of time renders the migration at best a very imperfect means of adaptation for surroundings in which light and darkness follow each other at half-day intervals.

#### V SUMMARY

In both *Palæmonetes* and *Cambarus* the proximal retinal pigment migrates distally when the temperature is lowered and proximally when it is raised.

In *Palæmonetes* the distal pigment migrates proximally when the temperature is lowered, and distally when it is raised.

In all cases increased temperatures cause a pigment movement the reverse in direction to that produced by increased light.

The effect of temperature is much weaker than that of light.

In the eyes of crustaceans retinal pigment migration due to temperature changes is probably not adaptive.

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## OBSERVATIONS AND EXPERIMENTS ON REGENERATION IN LUMBRICULUS<sup>1</sup>

BY

S. MORGULIS

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This paper is the outcome of some experiments carried out mainly during the months of last autumn. The problem was suggested to me by Prof. T. H. Morgan, under whose direction this work has been done, and it gives me great pleasure to avail myself of this opportunity to express my sincerest gratitude to Prof. T. H. Morgan for having awakened in me an active interest in the subject, and also for his kindness in revising the manuscript.

### INTRODUCTION

The worms, 4–5 cm. long, are made up of 100–180 or even more segments, each bearing four pairs of setæ. The first seven to eight segments are readily distinguished from the rest of the body, and to them is applied the name “head.” A median dorsal blood vessel gives off in each segment a pair of lateral branches, and also a pair of blunt finger-like diverticula, called the “blood glands,” which are absent in the first nine or ten segments.

<sup>1</sup> This is the species originally described by Leidy as *Lumbriculus limosus*, and kindly identified for me by Dr. Percy Moore, as *Thinodrilus limosus*.

The worms were found along the edges of ponds between decaying leaves. In aquaria they can be seen on the bottom with their tails deeply embedded in the mud, and with the anterior parts of the body extending out like poles, always at an acute angle with the level of the bottom. When the water is disturbed, or when they are approached by a pipette, they instantaneously disappear under the mud.

To mechanical stimuli, such as irritation by means of a sharply or bluntly pointed object, *Lumbriculus* responds more or less readily: the more anteriorly the stimulus is applied the more rapidly and vigorously the worm reacts. No reaction, or only a very slight one, is apparent when the tail is touched. If the anterior end is stimulated by a current of water or by repeated tickling with a needle, the worm remains quiet and motionless for a while. This quietness may be easily misinterpreted as a failure to perceive the stimulus, but such a conclusion would be erroneous. Prof. C. O. Whitman gives a very interesting account of a similar, but very much more striking kind of reaction in *Clepsine*.<sup>2</sup> He points out that if the surface of the water is touched with the point of a needle just above the animal's back, it ceases to respire and becomes quiet, aptly called by Prof. C. O. Whitman—deceptive quiet. That the animal, although motionless, is in a state of active resistance, is shown by the rigidity of the musculature and some other symptoms. In the case of *Lumbriculus* I could not actually see the strained condition of the musculature, but the stretching out of the setæ is probably an indication of such a condition.

*Clepsine* in the condition of "active resistance" adheres to the dish so firmly that it is very difficult to force its hold. "With one end detached, the other will often hold against a pull strong enough to snap the body in two." I have observed similar cases in *Lumbriculus*. When stimulated, the worm sometimes becomes so firmly attached to the surface of the leaf, that it is impossible to draw it into the pipette, although a part of its body may be broken off by the forcible current of water in and out of the pipette. This,

<sup>2</sup> Prof. C. O. Whitman: *Animal Behavior*. Biol. Lectures.

however, happens very rarely. *Lumbriculus* also shows a thigmotactic reaction. In dishes of water the worms show a tendency to crowd together, rolling up in a ball, at times of considerable size, containing as many as twenty to thirty worms.<sup>3</sup>

Separate pieces taken from different levels also show the same reaction to mechanical stimuli as do the corresponding regions of the normal worm. Only the anterior pieces however come together to form balls.

#### AUTOTOMY OR REPRODUCTION

It is well known that of all the Oligochaeta *Lumbriculus* possesses the greatest capacity of breaking off pieces of their body when subjected to stimuli. "Wie schon gesagt, sind die Thiere (*Lumbriculus*) äusserst empfindlich gegen äussere Reize und zerreißen sich häufig schon bei ganz leiser Berührung an der getroffenen Stelle. . . . Deshalb findet man so häufig im freien Wasser, noch mehr unter den in Gläsergefässen gezüchteten Thieren, verstümmelte Individuen, da sie beim Anstossen an die Wände oder sonstige harte Gegenstände leicht zerbrechen."<sup>4</sup>

O. F. Müller<sup>5</sup> also speaks of this behavior. If, he says, *Lumbriculi* be put into dishes—"so wird man bald an ihnen den Schwanz vermissen; selbst in ihrem natürlichen Aufenthalt trifft man wenige unbeschädigt an; die meisten sind in Begriff, einen neuen Schwanz, andere einen neuen Kopf, noch andere beides zu entwickeln . . . . Demnach scheint dieses Zertheilen ihnen natürlich zu sein, und vielleicht das Mittel der Erhaltung ihrer Art."

In Table I, I have summarized Bülow's figures regarding the condition in which the worms are found in nature. These data were obtained from a very large number of individuals.

From this table we see at a glance how large is the proportion of individuals regenerating, especially those regenerating their tails,

<sup>3</sup> C. M. Child gives a similar description of the behavior of a fresh water nemertean *Stichostemma* "Several specimens in a jar of clear water will often aggregate in a single mass, crawling over and between each other and finally becoming nearly quiet." *The Habits and Natural History of Stichostemma*, Amer. Natur., xxxv, 1901.

<sup>4</sup> O. Diefenbach: *Anatomische und systematische Studien an Oligochaeta limicolae*. 24 Ber. Oberhess. Ges., Giessen, 1886.

<sup>5</sup> O. F. Müller: *Von Würmern des süßen und salzigen Wassers*, Kopenhagen, 1771.



which justifies Bülow's question—"ob denn allen Thieren mit irgend welchen regenerirten Enden die einst verlorenen Stücke von Feinden abgerissen seien, um als deren Nahrung zu dienen, oder ob nicht etwa der Lumbriculus bei seiner eminent weitgehenden Regenerationsfähigkeit sich selbst verstümmelte, d. h. in Stücke reisse oder zerfalle um aus diesen Stücken ganze Thiere entstehen zu lassen und auf diese Weise durch einfache Quertheilung, also ohne vorher angelegte Knospungszone, sein Geschlecht fortzupflauren."

Bülow decides the question in favor of the latter possibility. Müller, as we have seen, is inclined to give a similar interpretation. On the other hand this general view is disputed by some observers. Thus Dffenbach accounts for the breaking up as the result of the attacks of enemies. In order to throw some light upon this ques-

TABLE I

Month	Individuals with regenerating heads	Individuals with regenerating tails	Individuals with regenerating heads and tails	Non-regenerat- ing individuals
July, per cent.....	15.6	42.5	32.7	5.8
September, per cent.....	22	40	25	9

tion, *i.e.*, to test whether the breaking up is merely a case of "reflex throwing off of parts of the body, or autotomy,"<sup>6</sup> or whether it is to be looked upon as a normal process of reproduction, the following observations were made.

I found that in the American form of *Lumbriculus* there is lacking the high degree of sensibility described by almost all students of the European form. Worms kept in any kind of a dish, in the presence or absence of mud, never broke up into pieces. Drawing them into a pipette, and then spurting them out in a strong current of water, repeated many times in succession, never caused them to break apart in any way; nor did they react when put out on a paper or in the palm of my hand. To be sure, care must be taken not to injure the extremely delicate body-wall of the worms,

<sup>6</sup> Prof. T. H. Morgan: *Regeneration*, p. 110, 1901.

as this may result in a splitting off at the point of injury. But even in such a case the splitting is by no means a direct result to the irritation since the pinching in two is very slow, and requires sometimes hours for its completion.

I tried to imitate the attack of an enemy by grasping either end of the worm, and letting the other end hang down. The suspension of the worms in such a position for several minutes gave absolutely no results.

Neither could I verify Müller's statement that a breaking off of portions of the worm's body is effected by the addition of alcohol to the water. According to my observations alcohol as well as some other substance effects a breaking through of the body-wall at various points, and a consequent loss of blood through the broken blood vessels, but these ruptures of the body are never deep enough at the start to bring about the separation of portions of the worm, and if the worms be brought back into pure water, do not proceed any further. As a matter of fact some worms may recuperate perfectly from this treatment. The same thing will happen if the worms be subjected to drying. The body-wall will actually burst open in many places, but if put back into water in due time, the worms may remain intact and continue to live.

These results, I think, show pretty conclusively that the breaking off of parts of the worms is in some way or another connected with an injury to or distortion of the body-wall and is by no means the expression of a reflex action.

This difference in behavior of the European and American *Lumbriculus* may appear to be due to a difference in species, were it not that v. Wagner, a careful student of *Lumbriculus*, throws some doubt upon the remarkable behavior ascribed to the European species. "Ich habe schon 1893 mitgetheilt, dass es mir, trotzdem ich seit Jahren Lumbrikeln halte, nicht gelang, den spontanen Zerfall derselben beobachten zu können; ich möchte die Thiere noch so unsanft behandeln, niemals reagirten dieselben durch plötzliches Zerbrechen . . . . Seither ('00) habe ich mich, wenn auch mit wiederholten Unterbrechungen, doch weitere Jahre mit der Haltung und Beobachtung von Lumbrikeln befasst,

und ich kann wieder neuerlich sagen, *das mir die Erscheinung der Selbstzerstücklung niemals vorgekommen ist.*"

To the same effect we read in Mrázek's recent paper—"Wenn es nach den Ausführungen v. Wagner's noch eines weitem Beweises bedürfte, dass die Lumbrikeln keineswegs allzu empfindlich sind gegen mechanische Reize, so würden meine Erfahrungen einen solchen in hinreichendem Masse liefern."

If, then, the pinching apart is not a case of autotomy, let us consider whether the pinching off of pieces of the worms is a mode of reproduction. The strongest evidence in favor of this view is Bülow's record of the number of regenerating individuals found in nature, referred to above, and his own experiments, which may be summed up thus: out of twenty-five worms, handled with utmost care, in course of five to six weeks, fifteen worms have divided into fifty-one pieces, which gives an average of 3.5 piece to each worm. Other observers, as v. Wagner, for instance, favor this view simply because at a certain period they found the number of worms in the aquaria increased, the worms being at the same time smaller. It is to be regretted that Bülow does not state definitely how much each of the worms classified as "individuals regenerating their tails" was actually regenerated.

To fill up this gap I have recorded the amount of regenerated tissue in worms found in their natural environment. The number of worms was unfortunately much smaller than that used by Bülow. The measurement given in Table II do not pretend to be exact, and, indeed, were made rather roughly, nevertheless they will serve sufficiently for my purpose to show the size relations between the old and new tissue.

This table shows that most of the worms that might be referred to as "individuals with regenerating tails" regenerate in reality only the tips of the tails. The ease with which the worms lose this portion of their body is due to the exceedingly delicate body-wall of this region. "Der Körper ist ungemein mürbe, so dass namentlich das Hinterende leicht abbricht, wird aber in kurzer Zeit regenerirt."<sup>7</sup>

<sup>7</sup> Fr. Vejdovsky.

If we should disregard this slight amount of regenerated tissue, and group all these individuals as "nonregenerating," the whole aspect of conditions will be utterly changed. There would be

TABLE II

Length in cm.	Part missing	Part regenerating	General remarks
3.5			Was injured, pinched off two hours later,
4.5		Tip of tail, 3 mm.	Was injured, pinched off (noticed only next morning).
3.5		Tip of tail, 3 mm.	
5.		Tip of tail, 4 mm.	
4.	Tip of tail.		
4.5	Very tip of the tail.		
4.5		Tip of tail, 7 mm.	
4.		Tip of tail, 7 mm.	
4.5		Tip of tail, 5 mm.	
3.5	Very tip of the tail.		
4.5		Tip of tail, 4 mm.	
3.5	Very tip of the tail.	Head	
4.		Tail, 1.5 cm.	Could not determine with surety that the tail was really regenerated.
4.	Very tip of the tail.		
4.	Very tip of the tail.		
4.		Tip of tail, 6 mm.	
3.5			
4.	Tip of the tail.	Tip of tail, 5 mm.	
5.		Tip of tail, 6 mm.	
2.5		Tip of tail, 5 mm.	
3.		Tip of tail, 6 mm.	
4.5		Tail, 1.5 cm.	The tip of the tail consists of two to three zones of regenerated tissue.
3.5	Very tip of the tail.	Tip of tail, 8 mm.	
3.		Tip of tail, 5 mm.	
5.			
3.		Tail, 1.5 cm.	
3.		Head and Tail, 1.5 cm.	The regenerated tail had its tip regenerating anew.

3.7 per cent regenerating heads, 11.1 per cent regenerating tails (this number may be even larger); 3.7 per cent regenerating tails and heads, and 81.4 per cent of "nonregenerating."

In the two cases, where the heads had regenerated, the size of the worms did not differ sufficiently from other intact worms, and there is no reason to suppose that these worms have necessarily resulted from a spontaneous division somewhere about the middle of the body. More likely the foremost few segments were lost by a mere accident.

Could any of the small pieces give rise to new worms and in this way propagate the species? I will show later that such pieces, unless large, will soon die. Moreover if the worms always divided in the middle of their body, we should expect to have as many worms with regenerating heads as those with regenerating tails, but this does not occur. A regenerated head has certain peculiarities that distinguish it from a normal head, that can be detected long after its completion. If it be true that the posterior pieces generally perish what good would such a process of division be for the continuation of the race.

As to the evidence brought forward by v. Wagner, I can only say that his statement is too uncertain, for, the presence of a large number of smaller worms might be as much an indication of larval development as of an asexual multiplication.

#### EXPERIMENTS

##### *A Smallest Part Capable of Regeneration*

Bonnét found that when he had divided a worm into sixteen pieces, each piece formed a perfect worm again. If we assume 120 segments as the average number for *Lumbriculus*, these pieces would have contained seven to eight segments each. In my own case a number of very small pieces one to five segments each were first tried.

In order to keep the smallest pieces alive, I first cut the worms into large pieces and allowed the cut surfaces to heal over for eighteen to twenty-four hours, and then cut off smaller parts near the closed ends. By using this method I was enabled to keep alive about one-third of the entire number of pieces for a time long enough to give results.

*Experiment I* (October 14). A number of pieces were cut off and allowed to heal for about eighteen hours. Then still smaller pieces were cut off near the closed ends.

*a* From the anterior half of the worms I obtained: two pieces of five segments each; five pieces of four; eight pieces of three; six pieces of two; and two pieces of one segment each.

Most of these pieces were found dead about the second day after the operation. On October 19, the survived pieces, containing 5, 4, 4, 4, 3, 2, 2 old segments, had all regenerated a head and a tail.

*b* From the posterior halves of the same worms, I obtained: five pieces of five segments each; eight pieces of four; nine pieces of three; two pieces of two; and two pieces of one segment each.

October 19, the surviving pieces of 5, 5, 5, 4, 4, 4, 4, 3, 3, 3, 3, 2, 2 had all regenerated a head and a tail.

This experiment shows that pieces containing only two segments are capable of regenerating.

*Experiment II. a* By following the same method, pieces from the anterior half of the worms, consisting of 3, 3, 3, 3, 2, 2, 1, 1, 1 old segments also produced a new head and tail.

This experiment showed very distinctly that a single segment is capable of maintaining its existence, and of regenerating a perfect head, consisting, as is usually the case, of six segments, and also a tail.

*b* From the posterior halves of the same worms, I got thirteen pieces of three segments each; sixteen of two segments and twelve of one segment. Of these there regenerated eight pieces of three segments, seven pieces of two segments each, and none consisting of one segment only.

### *B Rate of Posterior Regeneration*

The object of the following experiments was to determine whether the length of the piece or its relative position in the worm's body is directly responsible for the rate of its posterior regeneration.<sup>8</sup>

*Experiment III* (October 25). About thirty-five worms were divided into seven parts each. The seventh piece (the tip of the

<sup>8</sup> Archiv. f. Entwicklungsm., xiv, p. 586, 1902.

tail) was not utilized in these experiments. Although the average length of these terminal pieces was somewhat over forty to forty-five segments they all died.

Pieces of corresponding levels were kept in the same dish, so that they were all practically under like conditions. According to the level to which the pieces belong, they will be named  $A_1$ ,  $A_2$ ,  $A_3$ ,  $A_4$ ,  $A_5$ ,  $A_6$ . At the end of two weeks all the tails that had been regenerated by these pieces, were cut off; the number of their segments as well as the number of segments in pieces, by which they were produced, was recorded, and the results are given in Table III. In this table is also given the average number of regenerated segments per one old segment (in the last line). By this method of calculation all the individual variations were obliterated, and at the same time this, so to speak, "ideal old segment" with the corresponding "ideal" number of new segments served to indicate the regenerative power at a given level, and served also as a basis for the comparison of the rates of regeneration.

In accordance with our method of calculation, we find that to each of the original segments at the first level, there were on the average 3.2 new segments; at the second level 3.3, at the third level 3.1, at the fourth level 2.6, at the fifth level 1.8, and at the sixth level only 0.9 new segments.

There is no evidence of a correspondence between the number of old segments and the number of new, regenerated segments, provided the old parts are not very different in length. Glancing over the Table III we can see at once that in pieces at the first level, those having twelve segments produced thirty-two or fifty new ones; those having thirteen old segments thirteen or forty-two. In pieces at the second level also, those of eleven old segments regenerated twenty-nine or fifty-six, and those of sixteen may regenerate eighteen or fifty-four new segments, etc. The same lack of correspondence will be found in all parts of this table as well as in subsequent ones.

On the other hand if we compare the smallest pieces of a certain level with the largest pieces of the same level we may sometimes find very considerable differences in the amount of regener-

ation. Thus in pieces of the third level those of seven or nine segments regenerate thirty-five or thirty-eight segments respectively, whereas pieces of twenty-eight, twenty-nine and thirty-one

TABLE III  
October 25, 1906/November 8, 1906

A <sub>1</sub>		A <sub>2</sub>		A <sub>3</sub>		A <sub>4</sub>		A <sub>5</sub>		A <sub>6</sub>	
No. of segments in old part	No. of segments in new tail	No. of segments in old part	No. of segments in new tail	No. of segments in old part	No. of segments in new tail	No. of segments in old part	No. of segments in new tail	No. of segments in old part	No. of segments in new tail	No. of segments in old part	No. of segments in new tail
10	22	8	25	7	35	11	42	8	40	11	10
11	32	10	45	9	38	12	31	11	28	16	17
12	38	10	46	10	41	12	49	14	20	18	15
12	42	11	29	10	41	12	62	16	38	18	24
12	44	11	35	10	54	15	45	17	42	20	8
12	50	11	36	11	28	15	46	17	56	20	16
13	13	11	47	11	52	15	58	19	0	22	22
13	32	11	48	13	0	18	34	19	44	21	15
13	36	11	56	13	37	18	42	20	34	21	23
13	42	12	50	13	44	19	50	20	42	24	10
14	44	12	65	14	50	19	50	21	38	25	36
14	53	13	0	14	56	19	80	22	21	25	48
14	55	13	42	14	66	20	45	22	35	28	18
14	56	14	42	15	50	20	49	22	40	30	9
15	48	15	66	16	58	20	55	22	48	30	40
15	57	15	70	16	60	20	58	23	43	32	30
16	40	16	18	17	47	20	60	25	21	42	32
20	58	16	49	17	48	21	0	25	42	45	27
21	68	16	54	18	55	22	38	26	42		
23	64	17	61	19	32	22	52	28	28		
25	90	18	52	21	53	22	53	28	42		
		19	66	28	68	22	54	28	58		
		24	65	29	60	23	49	30	45		
		26	65	31	77	24	55	30	50		
						26	66	31	37		
						30	55	36	76		
3.2		3.3		3.1		2.6		1.8		0.9	

segments regenerate as many as sixty-eight, sixty or ninety new segments.

It is not in the least surprising, I think, that pieces so *consider-*



ably different in size should have different regenerative capacities; still these differences are of little account when we take into consideration the regenerative capacity of pieces intermediate in size. Thus a piece of only fourteen segments belonging to the same level of the worms regenerated sixty-six segments, or six segments more than the piece of twenty-nine segments.

In view of this evidence we may safely assume that the length of a piece has no direct relation to the rate of its regeneration, and that the rate is dependent upon the position of a piece in relation to the worm's head. The pieces nearer to the head have the highest regenerative power, which gradually decreases as we pass from the front backward.

I wish also to point out in this connection the great range of variability in the regenerative power of various worms, that can be easily seen on looking over every column of the table.

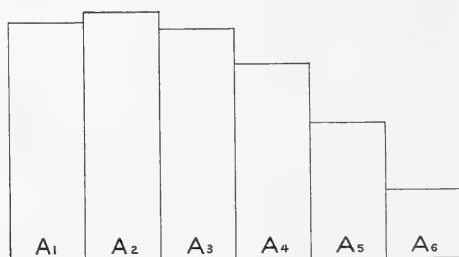


Diagram showing the rate of regeneration at different levels.

In the accompanying diagram an attempt is made to demonstrate the rate of regeneration at different levels by means of rectangles. The horizontal lines represent the "ideal" old segments of different levels, whereas the vertical ones represent the "ideal" number of new segments, regenerated at these levels. In either case a segment is expressed by a line 1 cm. long.

The worms were kept in clear, filtered water, so that they did not have any food for two weeks, but since the pieces had no heads they would not have been able in any case to feed for a greater part of the time.

After fourteen days (November 21) the pieces A<sub>1</sub> to A<sub>6</sub> were examined again. It will be remembered that on November 8,

the regenerated tails of these pieces had been cut off, so that since then the worms had been regenerating anew. Only pieces of three levels ( $A_3$ ,  $A_4$ ,  $A_5$ ) were alive. The data are given in Table IV.

TABLE IV  
November 8/November 21

$A_3$		$A_4$		$A_5$	
No. of segments in old part	No. of segments in new tail	No. of segments in old part	No. of segments in new tail	No. of segments in old part	No. of segments in new tail
9	16	9	16	6	12
9	33	11	27	11	14
10	15	12	6	12	4
11	24	13	34	15	22
12	10	14	24	15	23
14	22	15	46	15	25
14	27	16	22	18	0
14	32	17	17	18	20
14	38	17	22	18	21
15	25	17	28	18	22
15	32	18	20	19	17
16	26	18	22	20	6
16	34	19	37	20	9
17	28	20	20	20	12
19	26	20	26	21	26
26	41	20	27	23	28
27	43	20	29	24	13
29	45	20	33	24	22
		21	24	25	40
		24	44	26	26
		30	36	27	27
				28	17
				36	49
1.8		1.5		1.0	

This table shows that in course of the second period of two weeks, the pieces regenerated about one-half as many segments as were regenerated for the first period of two weeks. The regenerated tails were cut off once more, November 21. (It should be remarked in this connection that not only the regenerated tails, but also the regenerated heads as well were cut off every time.)

After a third period of two weeks (December 5) the pieces  $A_3$ ,  $A_4$  and  $A_5$  were examined. The result is recorded in the Table V.

Again we see that the pieces have regenerated only about one-half as many segments regenerated for the second period of two weeks, and about one-fourth as many for the first two weeks.

TABLE V  
*November 21/December 5*

$A_3$		$A_4$		$A_5$	
No. of segments in old part	No. of segments in new tail	No. of segments in old part	No. of segments in new tail	No. of segments in old part	No. of segments in new tail
9	16	10	2	12	2
10	6	10	9	13	12
11	7	14	3	15	10
12	0	14	10	17	7
13	0	16	7	17	9
13	9	16	9	18	4
14	12	18	22	18	5
15	10	19	13	19	6
15	18	21	3	19	13
16	11			20	17
16	15			22	10
17	15			23	14
24	21			24	13
26	31			27	5
29	27				
0.8		0.6		0.5	

It is worth mentioning in this connection, that the ratio between the amounts of regeneration at various levels remains almost constant during the three successive periods of two weeks each. Thus the ratio between the amounts of regeneration of  $A_3$  and  $A_4$ ,  $A_3$  and  $A_5$ ,  $A_4$  and  $A_5$ , for the first period will be:

$$\frac{3.1}{2.6} = 1.2; \quad \frac{3.1}{1.8} = 1.7; \quad \frac{2.6}{1.8} = 1.4$$

The same for the second period will be:

$$\frac{1.81}{1.5} = 1.2; \quad \frac{1.8}{1.0} = 1.8; \quad \frac{1.5}{1.0} = 1.5$$

And for the third period:

$$\frac{0.8}{0.6} = 1.3; \frac{0.8}{0.5} = 1.6; \frac{0.6}{0.5} = 1.2, \text{ or}$$

TABLE VI

	November 8	November 21	December 5
A <sub>3</sub> :A <sub>4</sub> .....	1.2	1.2	1.3
A <sub>3</sub> :A <sub>5</sub> .....	1.7	1.8	1.6
A <sub>4</sub> :A <sub>5</sub> .....	1.4	1.5	1.2

*Experiment IV.* Another experiment was started on November 29. A number of worms were cut into seven pieces each, as in the previous experiment. Pieces of corresponding levels were kept in the same dish. The dishes contained nothing but filtered water. No food was present. The pieces will be named according to the level of the body B<sub>1</sub>, B<sub>2</sub>, B<sub>3</sub>, B<sub>4</sub>, B<sub>5</sub>, B<sub>6</sub>.

The worms used in this experiment had been in the laboratory for some weeks, so that their regenerative capacity was considerably lessened. In general worms just brought in from the pond are the best as far as their regenerative power is concerned, and lose it when kept in the laboratory.

I regret that I did not take the record of the amount of regeneration at the end of the first two weeks, as it would be very interesting in connection with the results obtained from this experiment.

At the end of four weeks (December 27) the pieces were examined, the segments counted, and their numbers are given in Table VII.

This table shows practically the same result in regard to the relation of the regenerated to the old tissue, and that of the former to the level of the worm's body, as the Tables III, IV and V.

In this case, however, the regenerated tissue was not removed, and the pieces were left regenerating till January 10, 1907, or for a total of six weeks. Table VIII gives the result of counting the segments at the end of six weeks.

The number of new segments is the same as at the end of four

weeks, *i.e.*, no new segments had regenerated posteriorly. The regenerated segments, however, grew larger, and all the microscopical ones at the tip of the tail became conspicuous, and their setæ of considerable size. Slight differences in both these tables (VII and VIII) are probably due to some miscounts, which are almost inevitable.

TABLE VII  
November 29, 1906/December 29, 1906

B <sub>1</sub>		B <sub>2</sub>		B <sub>3</sub>		B <sub>4</sub>		B <sub>5</sub>		B <sub>6</sub>	
No. of segments in old part.	No. of segments in new tail	No. of segments in old part	No. of segments in new tail	No. of segments in old part	No. of segments in new tail	No. of segments in old part	No. of segments in new tail	No. of segments in old part	No. of segments in new tail	No. of segments in old part	No. of segments in new tail
11	25	10	21	10	17	13	13	15	13	16	7
12	20	11	22	11	19	13	20	16	0	16	15
12	40	12	24	12	30	13	30	16	21	17	5
13	35	13	26	12	30	13	32	16	23	20	12
14	35	13	32	14	12	16	18	17	11	23	28
15	26	13	36	14	26	16	34	17	15	24	7
15	40	14	36	15	39	16	39	17	15	24	13
16	31	14	40	16	22	17	27	17	18	29	18
16	40	15	26	16	43	17	28	18	17		
17	30	15	33	17	21	18	26	18	21		
17	32	15	44	17	34	18	32	19	12		
17	45	18	36	17	39	20	30	19	42		
18	30	18	43	24	24	20	46	20	15		
18	38	18	45	24	40	22	25	21	17		
18	45	20	32			22	42	21	21		
18	45	21	50			23	35	21	30		
		8	15			27	44	21	35		
								22	25		
								23	26		
								24	36		
								27	26		
2.26		2.26		1.80		1.71		1.09		0.62	

Let us now consider this last experiment in connection with the previous one, and see what we can infer from them. In either case the pieces of the worms were regenerating without food for a period of six weeks. In the latter case, where the process was

going on undisturbed, the parts formed a certain number of segments, then the formation of new segments stopped, and only growth, or increase in size took place. It seems as if these pieces of worm, brought out of equilibrium by the operation, completed themselves, attained again a state of equilibrium, and formed

TABLE VIII

November 29, 1906/January 10, 1907

B <sub>1</sub>		B <sub>2</sub>		B <sub>3</sub>		B <sub>4</sub>		B <sub>5</sub>		B <sub>6</sub>	
No. of segments in old part	No. of segments in new tail	No. of segments in old part	No. of segments in new tail	No. of segments in old part	No. of segments in new tail	No. of segments in old part	No. of segments in new tail	No. of segments in old part.	No. of segments in new tail	No. of segments in old part	No. of segments in new tail
11	25	10	21	10	16	13	13	15	13	16	7
12	20	11	22	11	19	13	18	15	16	16	15
12	40	12	22	12	30	13	30	16	0	20	12
13	35	13	26	14	12	13	32	16	21	23	28
14	35	13	33	14	26	16	18	17	11	24	7
15	26	13	36	15	37	16	34	17	15	24	13
15	40	14	35	16	22	16	40	17	15	29	18
16	40	14	36	16	43	17	27	17	18		
17	31	14	40	17	21	17	28	18	17		
17	32	15	27	17	39	17	30	18	21		
17	47	16	23	24	27	18	28	19	12		
18	30	18	36	24	40	18	32	19	45		
18	34	18	47			20	46	20	15		
18	38	18	49			22	27	21	17		
18	44	20	32			22	42	21	22		
19	42	21	52			23	36	21	30		
						27	44	21	35		
								22	25		
								23	24		
								24	36		
								27	26		
2.24		2.24		1.75		1.74		1.09		0.66	

dwarf worms. These were formed by pieces one-seventh the original length of the worms. If in accordance with Bülow's data each worm divides spontaneously on an average into four pieces (3.5 pieces being the actual average), worms only twice as big as

those obtained from my experiment, would result, and would also very likely remain dwarf worms.

If my view in regard to the formation of dwarf worms from pieces of *Lumbriculus* is true, the worms if divided would become continually smaller and smaller, till they would be reduced to single segments.

As a result of the continual cutting off of the regenerated tissue more new material was produced than when only one cut was made through the same level. Thus in the first experiment for the third period of two weeks there were to each old segment 0.5-0.8 of a new segment, while the pieces cut only once did not produce even a single segment for the same length of time.

### *C Regeneration of Regenerated Parts*

Tails that had been regenerating from parts of *Lumbriculi* for some time, were cut off so that none of the old tissue remained. Some of the tails had been regenerating for two weeks. After removal from the old part a new tail was allowed to regenerate in course of the following two weeks. These were also cut off.

a October 31. Thirty such regenerated tails were obtained from pieces belonging to the third level, and thirty regenerated tails from pieces of the fifth level, of worms that were divided into eight parts each. These tails had been regenerating since October 17. The tails regenerated by pieces of the third level we will call for convenience A, and those regenerated by pieces of the fifth level B.

These sixty tails were kept for two weeks, and when examined on November 14, eighteen A tails and thirteen B tails, or half the original number were alive. Those that survived regenerated new heads of five to seven segments, with one exception, of which I shall speak presently. The little worms contained on an average, probably, about forty-five to fifty-five small segments.

*In the course of two following weeks they did not produce any new posterior segments.* That this is really so can be ascertained with certainty because in the regenerating tails the terminal five to six segments, which are the youngest, are microscopical in size. Dur-

ing the time intervening between October 31 and November 14, all these microscopical segments increase in size very much, their setæ became large, and the blood vascular system quite conspicuous, as in older segments; but no more 'anlage' of segments have made their appearance. The last large segment was contiguous to the anal segment.

This result shows that when a new regenerated tail regenerates a head, and thus forms a little worm, although the segments grow larger, no new segments are laid down at the posterior end. Whether the same holds true also for non-regenerated posterior ends of *Lumbriculus*, I do not know, since in all cases when posterior ends were removed they died.

*b* November 14. Again twenty-eight regenerated  $A_1$  tails, and thirty  $B_1$  tails were obtained from the same pieces as the foregoing at the third and fifth levels, which had been regenerating from October 31 till November 14, or for two weeks.

November 28, I found alive out of fifty-eight tails ( $A_1$ ,  $B_1$ ) eleven  $A_1$  and seven  $B_1$ . They all had regenerated new heads, although the number of segments in these heads was in some cases only four. Thus about one-third of all the regenerated tails produced new heads, and eighteen very small worms of about twenty-five to thirty segments each were obtained. In this case also no new segments were laid down at the posterior end, but the segments became larger.

*c* November 14, I cut in two all the thirty little worms formed by the regeneration of *A* and *B* tails, in order to determine the rate of their posterior regeneration. The cut was made not quite in the middle, so that the posterior parts were somewhat longer than the anterior.

After a lapse of two weeks, November 28, twelve anterior pieces which came from *A*, and eight anterior pieces from *B*, were found alive and regenerating new tails. None of the posterior pieces, although bigger than the anterior ones, had survived. (In other experiments a small percentage of the posterior pieces regenerated heads.) On the whole a very fair percentage (about 65 per cent) of the anterior parts survived the operation and continued to regenerate the missing tails.



In the tables given below are recorded the number of segments present in the anterior pieces left after the operation of November 14 (*c*) and the number of new segments they had produced at the end of two weeks, November 28.

In accordance with our method of calculating the average number of new segments per one old segment, to define the regenerative power, we will have 0.11 segments standing for this power in pieces from A and 0.06 segments for that in pieces from B.

TABLE IX

*November 14/November 28*

Anterior parts from worms formed by regeneration of A		Anterior parts from worms formed by regeneration of B	
No. of old seg- ments	No. of new seg- ments	No. of old seg- ments	No. of new seg- ments
16	bud*	16	bud
16	bud	17	bud
19	3	19	bud
19	5	19	6
20	bud	20	bud
20	bud	23	4
20	4	26	bud
21	3	26	bud
22	5	20.5	1.3
23	4	Average No. of new segments per one old segment, 0.06	
24	bud		
25	3		
20.4	2.3		
Average No. of new segments per one old segment, 0.11			

\* Whenever the term "bud" is used, it indicates that an unsegmented portion in which the anus lies is produced only, but no segments are deposited between this regenerated organ and the old tissue.

Of course these data are so meager that it would hardly justify much speculation, but it seems to me nevertheless very suggestive. If we compare the rate of posterior regeneration in the little worms formed from the anterior parts of the regenerated tail pieces (A and B), we find that they stand to each other in a ratio very much like that of the rate of posterior regeneration in the old parts, from which the A and B tails originate.

The parts of the third level regenerated from October 17 to October 31 the tails A at an average of 4.4 new segments for each old segment; and those of the fifth level regenerated in the same time the B tails at an average of 2.6 new segments. During the next two weeks, from October 31 until November 14 they had regenerated at an average 2.4 segments and 1.7 segments, respectively. The ratio between these rates of posterior regeneration is:

$$\frac{4.4}{2.6} = 1.7, \text{ and } \frac{2.4}{1.7} = 1.4$$

The ratio between the rates of posterior regeneration of the little worms formed from A and B is:

$$\frac{0.11}{0.06} = 1.8$$

The eighteen  $A_1$  and  $B_1$  tails, which survived and had regenerated heads were also cut in two. This operation was performed on November 28, or two weeks after they had been separated from the old tissue.

At the end of two weeks again (December 12), only seven anterior pieces from  $A_1$  were alive. The fate of these seven pieces is shown in the following Table.

TABLE X

November 28 No. of old segments.	December 12 No. of new segments.
14	bud
14	bud
15	bud
16	bud
17	bud
18	bud
27	bud

This table shows that only the anus was formed, but no new segments were produced in these seven worms.

Other experiments gave similar results and need not be recorded here. It is evident that a regenerated tail is not only capable of regenerating a head, from its anterior cut surface, but also of

replacing its posterior part when cut in two, and that the property of regeneration passes over to the new tissue together with the protoplasmic material it is built of.

#### *D A Case of Heteromorphosis*

One of the thirty-one A tails of the previous experiment regenerated a tail in place of a head. This is the only indubitable case of heteromorphosis in *Lumbriculus* of which we have record. That this was a genuine tail and not merely a misformed head can be easily proved (1) by the number of regenerated segments, (2) by the position of the anal aperture; (3) by its "functional activities."

When an abnormal head develops it does not contain more than six to seven new segments. Here eighteen segments were regenerated. The segments gradually decreased in size and were microscopical near the distal end. This sequence of segments is characteristic of the tail. In the regenerating head on the contrary all the material is laid down first, and then its segmentation appears.

In this case the terminal aperture is not a mouth (which may sometimes assume such an abnormal position) because it is round and not triangular in form, and lies in a knob of indifferentiated material, as in the case of the anus. The best proof of its being a tail is the direction of the contractions of the blood vessels, dissepiments and entire musculature. Whereas in a head contraction takes place from before backward, here it was in the reverse direction, viz: in that characteristic for a tail. In the old tail the contractions were running in a direction opposite to that of the heteromorphic tail. The waves of contractions in both tails started at their distal end, ran toward each other gradually slowing down and vanishing altogether in the vicinity of the point of their union. The contractions in the old tail were more vigorous, but they never passed beyond the old part.

This heteromorphic tail developed from one of a number of pieces that had been kept in the same dish. It could not therefore be due to an external influence. In order to see whether the old tail would again produce a heteromorphic tail or a head, and also to see what the heteromorphic tail would regenerate, I severed

the heteromorphic tail from the old one, exactly at the line of their union. Unfortunately, both pieces soon died after the operation.

*E Some Comments on Anterior Regeneration*

Although this subject was pretty thoroughly studied by Bülow and v. Wagner, there are some points that have not been considered at all.

In the formation of the new head, abnormalities are not infrequent. Double heads, arising immediately from the cut surface; or from a common stalk a little distance from the cut surface occur in about 5 to 10 per cent of the pieces. At the posterior cut surface, on the contrary, double malformations are of very great rareness. The only instance I find recorded in the literature is that spoken of by Bülow, of a worm developing a double tail, and another similar instance which I found last summer. From the posterior cut surface there grew out two tails of somewhat different lengths, and the whole worm had a Y-shaped form.

By observing the process of regeneration of an abnormal head one will be impressed by the constant movements that are going on inside the body-walls in a forward direction. These exert a great pressure upon the delicate regenerated epidermis, causing it to protrude in many points. This action may be largely responsible for these malformations, for if, on the other hand, the malformations are supposed to be due to the operation, why should we not find abnormalities in the regenerating tail also? I have never observed any malformation of the head of *Lumbriculi* freshly caught in ponds. Another point that I wish to call attention to is the dissimilarity between a regenerated and a normal head. "Das Vorderende ist immer etwas grünlich oder grünschwartz, was von dem Pigmente herrührt, welches namentlich die den Darmkanal bedeckenden Drüsen erfüllt."<sup>9</sup> This green pigment is arranged segmentally in seven to eight very deeply colored bands, which give to the head a striped appearance. If from one to seven of the anterior segments are removed, the number removed will be restored, but the substituted segments lack the pigment, and are

<sup>9</sup> Fr. Vejdovsky.

perfectly transparent. If six to seven segments are cut off, so that one or two more stripes of the greenish pigment are left in the old tissue, the head will be perfectly repaired but not a single granule of the old green pigment will be seen in the new segments, though I watched it for four weeks.

Thus the two adjacent head segments, one from the old worm, filled with pigment, the other produced by the worm anew, pale and pigmentless, lie side by side.

Heads regenerating from any other level of the worm's body, far away from the pigmented region, are also without pigment. If worms under natural conditions do reproduce themselves by dividing into several pieces (four being the approximate average, calculated from Bülow's experiments), should we not expect from this, that the majority of the worms in nature would have heads without pigment? In fact, such worms with pigmentless heads are not very frequent.

It is true that after a lapse of a considerable time the pigment is formed anew in the regenerated head, and begins to develop from the distal end of the regenerated head. This delayed development of the pigment in the regenerated head needs however a more complete study.

#### REGENERATION AND ADAPTATION

The tip of the tails in *Lumbriculi* is almost always missing, or regenerating, which fact indicates that this portion of the worm's body is easily injured and that these injuries are of a very frequent occurrence. On the other hand the power of regeneration is very feeble in this particular region as compared with that in the more anterior and less frequently injured regions.

If we attempt to find a connection between these facts, we shall, contrary to Weismann's claims, reach the conclusion, already expressed in 1898 by Prof. T. H. Morgan in regard to the frequency of accidental injuries and the power of regeneration in the hermit crab, that "no such relation is found to exist."

This low capacity of regeneration in a region where regeneration is always going on, seems to contradict the view that the capacity

to regenerate is an "adaptation of the organism to definite demands made upon it by conditions of life," and that it is "not the outcome of primary qualities of the living substance," "not an inherent quality of the organism," as it also contradicts the view that it is due to an "adaptation produced by natural selection."<sup>10</sup>

SUMMARY

1 Pieces of *Lumbriculus* containing only a single segment are capable of regenerating both a new head and tail.

2 Regeneration from a posterior end takes place more rapidly in pieces from the anterior region of the body, and gradually decreases as the pieces are taken from the more posterior region of the worm.

3 A piece of a worm, when subjected to the operation of cutting a few times will produce more new tissue for the same length of time than when subjected to cutting only once.

4 No relation whatsoever between the number of old segments in a piece and its rate of regeneration can be found.

5 There is no relation between the available food and the rate of posterior regeneration at different levels of the worm.

6 In regard to its regenerative capacity each worm shows variations of its own.

7 Regenerated tails, when detached from the old part, are capable of regenerating new heads, but do not produce any new posterior segments.

8 Pieces of such regenerated tails are also capable of posterior as well as anterior regeneration, from the posterior and anterior cut surfaces.

9 The pigment of the regenerated head probably does not arise in connection with the old pigment, but develops anew.

10 In the case of the anterior regeneration, where only six to seven (eight) segments come back, the eighth (or ninth) to the tenth (or eleventh) segments of the old worm are dropped out.

11 The experimental evidence, likewise that from observations,

<sup>10</sup> A. Weismann: *The Germ-Plasm*, 1893.

is opposed to the view that the breaking off of pieces of the worms with their subsequent regeneration, is a regular mode of reproduction in *Lumbriculus*.

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# CORRELATION AND VARIATION IN INTERNAL AND EXTERNAL CHARACTERS IN THE COMMON TOAD (BUFO LENTIGINOSUS AMERICANUS, LE C.)

BY

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WITH SIX FIGURES AND TWENTY-TWO TABLES

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## I INTRODUCTION

The present study was undertaken with a view toward getting information first as to the degrees of correlation and variability of internal characters, such as the viscera and muscles, regarding which our knowledge is remarkably deficient, and second as to the general condition of correlation among a considerable number of characters in a single group of individuals. It has been pointed out frequently that selection acts not upon single characters or variations alone, nor as such, but upon the entire organism as the unit: that there results from this action a "balance of fitness"



among a large number of characters—in fact, throughout the entire organization. This condition of balance is to a certain extent measurable by coefficients of correlation which are merely indices of the corresponding change in a given character accompanying a certain change in another given character—"relative" and "subject." The ideal method of approaching this matter is of course through multiple correlations involving the relation of at least three characters but this method is not practicable because of the time involved in carrying through such calculations. Consequently the method adopted here is merely to calculate the coefficients of correlation between all of the measured characters in pairs.

The material from which the data were drawn was unusually favorable for such a study. Toads were readily secured, were not affected by capture and were easily kept in nearly normal conditions during the brief time elapsing between their capture and measurement. The group studied was a perfectly homogeneous one, all in excellent nutritional condition, with precisely similar environmental conditions, and quite isolated geographically. The number measured and weighed was not large absolutely (425) but the fact that practically an entire colony of the particular variety under observation was collected and measured is almost unique. The results are therefore at least free from errors due to the sampling of a larger population, errors which easily may be so great as to vitiate results even though a most careful preliminary study of the organism and its habits may have been made and particular care taken to procure a "random" sample.

The fact that the subjects were of various ages, the growth phenomena not being taken into account, renders the data given here of no value for certain special purposes, but for the relations which are being sought here this is not an objection. We are dealing here with the conditions of correlation and variation in a total natural society of normal individuals.

## II SUMMARY

The material under consideration consisted of practically an entire colony of the common toad. Accurate measurements were

taken of thirteen characters, including both external dimensions and internal organs. The numerical ratio between the sexes was found to be 658 males to 1000 females. The sexes are perfectly distinct with respect to size, variability and correlation. The females are on the whole about 24 per cent larger, 23 per cent more variable and 10 per cent better correlated than the males. The internal characters are about four times as variable as the external. The ratios between the average values of pairs of characters remain the same in the two sexes. The distributions are all skew and nearly all negatively, apparently the result of including individuals of all ages over one year.

The correlation coefficients are all relatively high. The external characters although less variable than the internal, are in the males 51 per cent and in the females 30 per cent better correlated than the internal characters. Those individuals above the average in any pair of characters show much less "scatter" about the regression line than those below the average.

In the general discussion these results are compared with those of other observers. Some of the points mentioned are the relation between efficiency and mass or dimensions in external and internal characters; the extremely high variability of internal characters; the relation between variability and correlation.

The conclusion is reached that from the side of fitness or survival conditions of correlation here seem to be more fundamental than conditions of variability, and that the general subject of correlation is of increasing importance.

### III THE DATA

#### I *Material*

The subjects from which these data<sup>1</sup> were secured consisted of a society of 441 individuals of the Common American Toad (*Bufo lentiginosus americanus*, Le. C.) The society was one found on Cedar Point, Ohio, a low sandy point 200 to 300 yards wide extending for six or seven miles obliquely into Lake Erie and

<sup>1</sup> The data were secured during the summer of 1905 at the Lake Laboratory of the Ohio State University.

partly enclosing Sandusky Bay. Toward the extremity of this point a colony of toads has become established the individuals of which differ in several minor respects from those of the main-land. The colony is fairly isolated geographically and inhabits the lake beach for a distance of about a mile and a half. During the daytime the toads lie buried about two inches below the hot surface of the sand and only a few feet or yards back from the water's edge. Shortly after sunset they uncover and hop down to the water to pick up food carried in by the usually light wash. Food is more than abundant throughout the season and with no particular exertion all are able to maintain themselves in an extremely well fed condition. As it becomes fully dark they assemble in this fashion, sometimes just within reach of the water and at the beginning of the season were picked up easily in considerable numbers. During the summer almost daily collections were made and toward the end subjects became so rare that ultimately only a dozen or two could be found during an entire week.

The data therefore were drawn from practically an entire population occupying a uniform stretch of sandy beach about twenty feet wide and a mile and a half in extent where all were subject to conditions which were remarkably uniform though somewhat unusual for creatures of their kind. And while the total number of individuals measured was not large absolutely, yet it represents nearly 100 per cent of this homogeneous group and consequently the observations are free from errors such as result from the sampling of a larger population.

## 2 *Methods*

All the toads one or more years old were collected: there is no way of determining their exact age. Since such characters as total weight or weights of viscera are liable to modification by the unusual conditions of confinement, care was taken not only to keep these conditions as nearly normal as possible but also to measure animals only recently removed from their natural surroundings. Immediately after collection in the evening the toads were placed in a large sand-box and no more were taken than could

be measured the following day. In no case was any measurement taken from an individual which had been more than twenty-seven hours in captivity.



Fig. 1 Ventral view of female toad in position for measurement. One-half natural size of toad weighing 41.0 grams.

The characters measured and methods of measurement are briefly as follows: (Compare with Fig. 1.)

1 Total weight. The toad was brushed clean and weighed alive. There was a gradual loss in weight during captivity chiefly due to defecation and evaporation of water. The rate per cent at which this loss occurred was determined in four series of different sized toads and the proper correction made for each individual according to hours elapsed from time of collection. Usually upon opening the abdominal cavity the bladder and alimentary canal were found empty; in the few individuals where this was not the case, proper correction was again made. The toad was then pithed (brain and cord) a broad incision being made to permit free loss of blood. While taking the succeeding measurements the toad was placed on its back and the blood drained off completely. For the next measurements the legs were placed in the position shown in Fig. 1.

2 Length of body. From tip of nose to end of body between thighs. This of course includes the head.

3 Length of thigh. One-half the distance between the middles of the knee-joints when in the position shown in the figure.

4 Length of shank. Distance from middle of knee-joint to middle of ankle-joint.

5 Length of foot. Distance from middle of ankle-joint to tip of longest (second) toe, when fully extended.

6 Length of leg. Sum of thigh, shank and foot.

7 Total length. Sum of body and leg.

8 Width of mouth. Transverse extent between angles of mouth when closed.

9 Length of head. Distance from tip of nose to postero-dorsal margin of cranium exposed by pithing incision.

10 Weight of gastrocnemii. The ankle-joint was completely flexed and the tendo Achilles sectioned in a radius of the joint. The attachment of the muscle to the femur was then cut along the bone and the muscle removed. Both muscles were weighed on a balance sensitive to one milligram. They were handled only with forceps, by the tendo Achilles and were not allowed to dry.

11 Weight of liver. The abdominal cavity was then opened and the liver removed by cutting through its attachments and blood-vessels closely along its surface. It was then gently rolled in a towel until it ceased to stain and weighed as above.

12 Weight of ovaries. These were removed in same manner as liver except that being practically bloodless, they were not rolled.

13 Length of alimentary canal. The trunk was then completely divided, the mesentery cut through and the alimentary canal straightened out to its full extent by pulling gently. A condition is soon reached where no farther stretching occurs and the length was measured when this point was reached. This character may be considered as little subject to error in determination as any visceral character, since a number of tests showed that this method of measurement gave a very reliable datum, much more reliable than was expected.

A résumé of the data will be given first as briefly as possible, discussion of their significance being deferred until they have been completely presented.

### 3 Numerical Ratio Between Sexes

Preliminary examination of the data shows at once that the sexes must be treated separately. Of the total number measured 173 were males and 252 females but these numbers are not quite indicative of the actual ratio, as sixteen additional individuals were collected and used for purposes that might have affected the values of some of the internal characters and which therefore were not measured. Of the total 441 collected 175 were males and 266 females, giving a ratio of 658 males to 1000 females in the entire colony.

### 4 Variability

The means and standard deviations of all the characters in the male and female series, calculated according to the usual methods<sup>2</sup> are given in Table I and the coefficients of variability in Table II.

<sup>2</sup>Formulae and methods from Davenport ('04).

$$\begin{aligned}
 M &= \frac{\Sigma (V.f)}{n} & E_M &= \pm 0.6745 \frac{\sigma}{\sqrt{n}} \\
 \sigma &= \sqrt{\frac{\Sigma (x^2.f)}{n}} & E_\sigma &= \pm 0.6745 \frac{\sigma}{\sqrt{2n}} \\
 C &= \frac{\sigma}{M} \times 100 & E_C &= \pm 0.6745 \frac{C}{\sqrt{2n}} \left[ 1 + 2 \left( \frac{C}{100} \right)^2 \right]^{\frac{1}{2}}
 \end{aligned}$$

TABLE I  
Means and standard deviations of all characters

	MEAN		STANDARD DEVIATION	
	♂ n=173	♀ n=252	♂ n=173	♀ n=252
Total weight, grams.....	33.540±0.364	52.560±0.617	7.105±0.257	14.526±0.436
Total length, mm.....	160.405±0.518	183.690±0.628	10.107±0.365	14.783±0.444
Length of body, mm. ....	67.871±0.221	78.113±0.292	4.319±0.156	6.877±0.207
Length of thigh, mm .....	29.575±0.101	33.690±0.098	1.970±0.071	2.538±0.076
Length of shank, mm.....	23.020±0.080	26.325±0.094	1.560±0.056	2.224±0.067
Length of foot, mm.....	40.840±0.133	46.506±0.155	2.594±0.094	3.657±0.110
Length of leg, mm.....	92.812±0.309	105.990±0.355	6.035±0.218	8.359±0.251
Length of head, mm.....	18.000±0.045	20.052±0.051	0.872±0.032	1.205±0.036
Width of mouth, mm.....	23.202±0.074	26.650±0.087	1.437±0.052	2.044±0.061
Length of alimentary canal, mm ...	353.873±1.779	429.603±2.253	34.670±1.255	53.018±1.593
Weight of gastrocnemii, decigrams..	6.832±0.086	10.635±0.131	1.673±0.060	3.077±0.092
Weight of liver, grams.....	1.590±0.027	2.233±0.034	0.530±0.019	0.794±0.024
Weight of ovaries, grams.....		5.253±0.209		2.795±0.145

TABLE II  
Coefficients of variability

	♂ n=173		♀ n=252	
	C	E <sub>c</sub>	C	E <sub>c</sub>
Total weight.....	21.18±0.80		27.64±0.89	
Total length.....	6.30±0.23		8.05±0.24	
Length of body .....	6.36±0.23		8.80±0.27	
Length of thigh.....	6.66±0.24		7.53±0.23	
Length of shank.....	6.78±0.25		8.45±0.25	
Length of foot.....	6.35±0.23		7.86±0.24	
Length of leg.....	6.50±0.24		7.89±0.24	
Length of head.....	4.82±0.17		6.01±0.18	
Width of mouth.....	6.20±0.22		7.67±0.23	
Length of alimentary canal.....	9.81±0.36		12.34±0.38	
Weight of gastrocnemii .....	24.50±0.94		28.94±0.94	
Weight of liver.....	33.34±1.33		33.55±1.20	
Weight of ovaries.....			53.23±3.39	

These tables show several facts of interest. First, the absolute distinctness of the male and female series as regards size, the female showing the larger values in every character measured. *On the whole the female is about 24 per cent larger than the male*, the actual

percentages varying from 11.4 in length of head to 56.7 in total weight. This same distinctness between the sexes was found in the calculation of all constants and consequently they were treated separately throughout.

Second, there is a corresponding distinctness between the males and females with respect to the amount of variability. The coefficient of variability may now be accepted as a thoroughly reliable measure of comparative variability. As expressed by this coefficient the females show a uniformly higher variability. *On the whole the females are about 23 per cent more variable than the males*, the actual percentages varying from 6.6 in weight of liver to 38.4 in length of body.

Finally, inspection of the coefficients of variability shows that the characters are separable into two quite distinct groups. The external dimensions show not only a comparatively low degree of variability but a remarkable uniformity, the limits of C being, with a single exception, 6.0 per cent and 8.8 per cent. The other characters, which we may call "internal" are several times more variable and show much less uniformity, the limits of C being 9.8 per cent and 53.2 per cent. Excluding the total weight, total length and length of leg as composite characters, we find that the average coefficients of variability of the external and internal characters are as follows:

	♂	♀
	Per cent	Per cent
External characters.....	6.2	7.7
Internal characters.....	22.5	32.5

That is, *the internal characters are roughly four times as variable as the external characters.*

It is an important consideration that, in spite of the very considerable disparity in absolute size of the two sexes, the ratios between the average values of pairs of characters are remarkably similar in male and female series. Table III gives a few of these ratios between characters measured in similar units. The ratios are extremely close throughout, particularly those between characters which are obviously quite closely related in function, the body and leg dimensions, for example, where the ratios are the same

through the second decimal place. This holds true for all of the external dimensional characters, namely, length of body, thigh, shank, foot, leg, head, width of mouth and total length. The ratio between weight of gastrocnemii and total weight is the same in both sexes but as related with the other characters the gastrocnemii are much lighter in the male. So too the liver is proportionately lighter in the male with respect to every character except total weight. The alimentary canal is, on the contrary, longer in the male except as compared with total weight, weight of gastrocnemii and liver. The ratio between total weight and other characters is quite inconstant: the males are lighter with respect to total length, length of body, foot, leg, head, and alimentary canal and heavier with respect to length of thigh, shank, width of mouth and weight of liver.

TABLE III

*Ratios between means of certain characters in the two sexes*

Ratio between average	♂	♀
Length of body and total length.....	.423	.425
Length of thigh and total length.....	.184	.183
Length of shank and total length.....	.143	.143
Length of foot and total length.....	.253	.255
Length of leg and total length.....	.578	.577
Length of head and total length.....	.112	.109
Total length and length of alimentary canal.....	.453	.428
Length of head and width of mouth . . . . .	.776	.752
Total weight and weight of gastrocnemii.....	.204	.202
Total weight and weight of liver.....	.474	.425

Upon plotting the measurements, examination of the frequency polygons shows that all of the distributions are skew. The exceptionally wide range of the measurements and the relative fewness of observations possible render any exact analysis of these curves unprofitable but one or two general points are to be noted. The curves are of the usual type given by such data (Pearson's Type IV) and in nearly all cases the skewness ( $\alpha = \frac{D}{\sigma}$ ) has a negative value, that is the greater number of individuals are below the average with respect to a given character. Table IV gives the means, theoretical modes and indices of skewness of all the characters measured. In all of the external characters, including total



weight, the skewness is negative and varies from  $-0.032$  to  $-0.241$  in the males and from  $-0.052$  to  $-0.458$  in the females. The skewness is positive in some of the internal characters—weight of liver male and female, and weight of gastrocnemii in male, in female the skewness here is negative but is hardly significant  $-0.010$ . It

TABLE IV  
*Skewness of the frequency polygons*

	♂				♀			
	Mean	Median	Theoretical mode	Skewness	Mean	Median	Theoretical mode	Skewness
Total weight.....	33.540	34.278	35.758	$-0.312$	52.560	52.812	53.318	$-0.052$
Total length.....	160.405	160.511	160.725	$-0.032$	183.690	185.948	190.464	$-0.458$
Length of body...	67.871	68.005	68.273	$-0.093$	78.113	78.935	80.578	$-0.359$
Length of thigh...	29.575	29.716	29.997	$-0.214$	33.690	33.930	34.410	$-0.288$
Length of shank...	23.020	23.055	23.126	$-0.068$	26.325	26.638	27.264	$-0.426$
Length of foot....	40.840	40.881	40.961	$-0.046$	46.506	47.000	47.988	$-0.405$
Length of leg.....	92.812	92.986	93.335	$-0.087$	105.990	107.231	109.713	$-0.445$
Length of head....	18.000	18.135	18.404	$-0.464$	20.052	20.151	20.351	$-0.250$
Width of mouth...	23.202	23.281	23.439	$-0.164$	26.650	26.900	27.400	$-0.368$
Length of ali. can.	353.873	356.662	362.240	$-0.241$	429.603	433.372	440.910	$-0.213$
Weight of gastrocnemii.....	6.832	6.766	6.635	$+0.118$	10.635	10.646	10.667	$-0.010$
Weight of liver....	1.590	1.565	1.517	$+0.136$	2.233	2.171	2.047	$+0.235$

should also be noted that in general the skewness of the females is the greater the average indices being  $-0.129$  in males and  $-0.292$  in females.

Apparently no data have been published showing the frequency polygons of visceral characters among any of the vertebrates except man. Figs. 2-4 are given therefore showing distributions of gastrocnemii, liver and alimentary canal, in male and female series, and for comparison Fig. 5, total weight, and Fig. 6, length of body, which is representative of the other external characters.

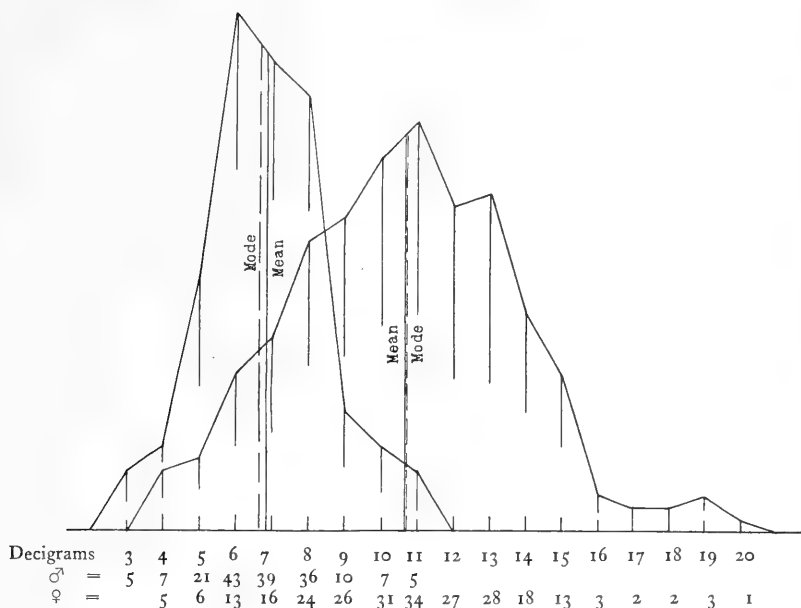


Fig. 2 Weight of both gastrocnemii.

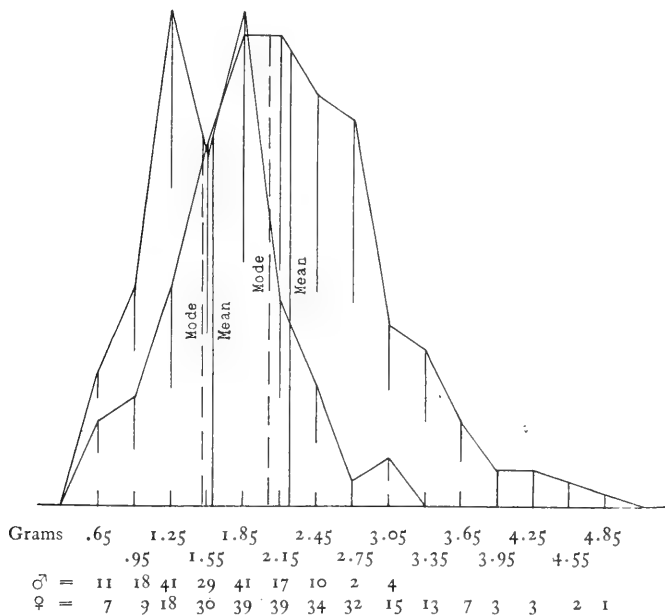
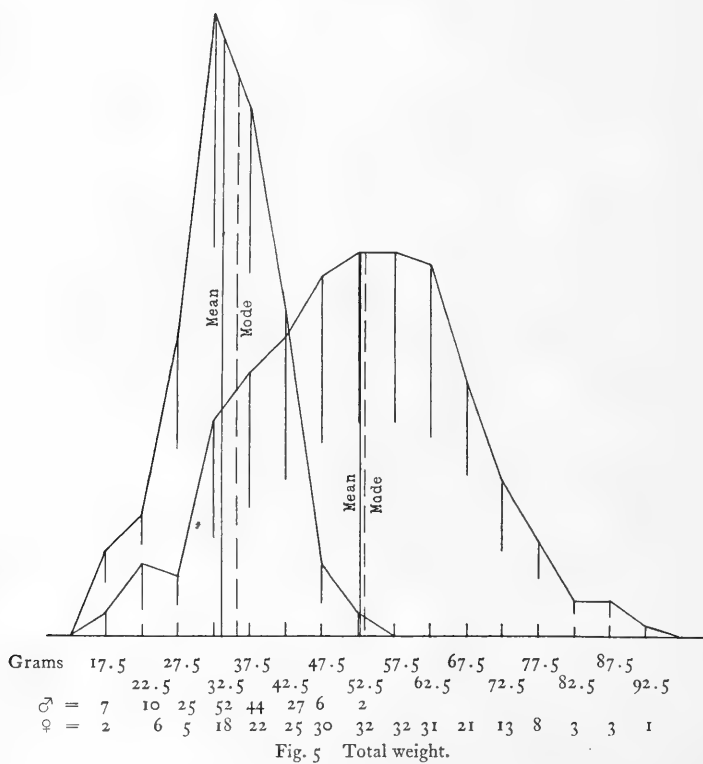
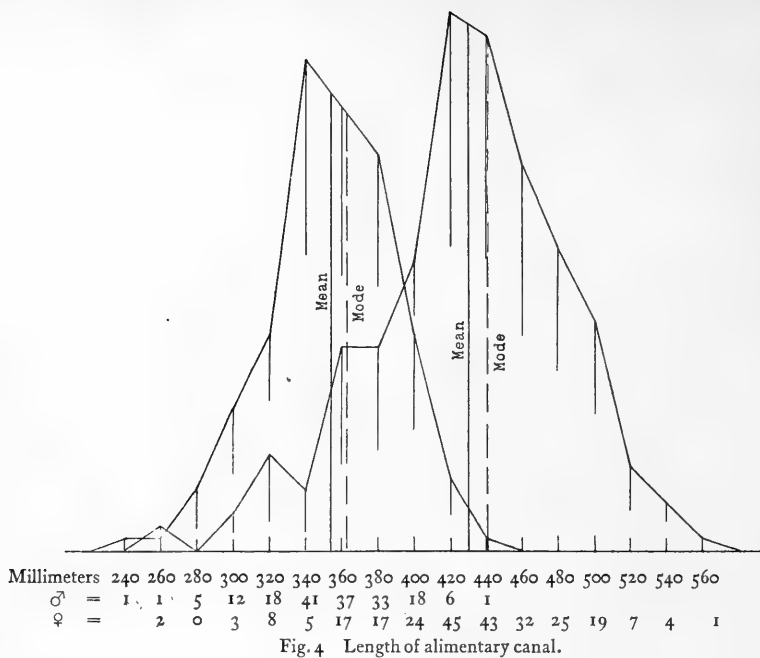


Fig. 3 Weight of liver.



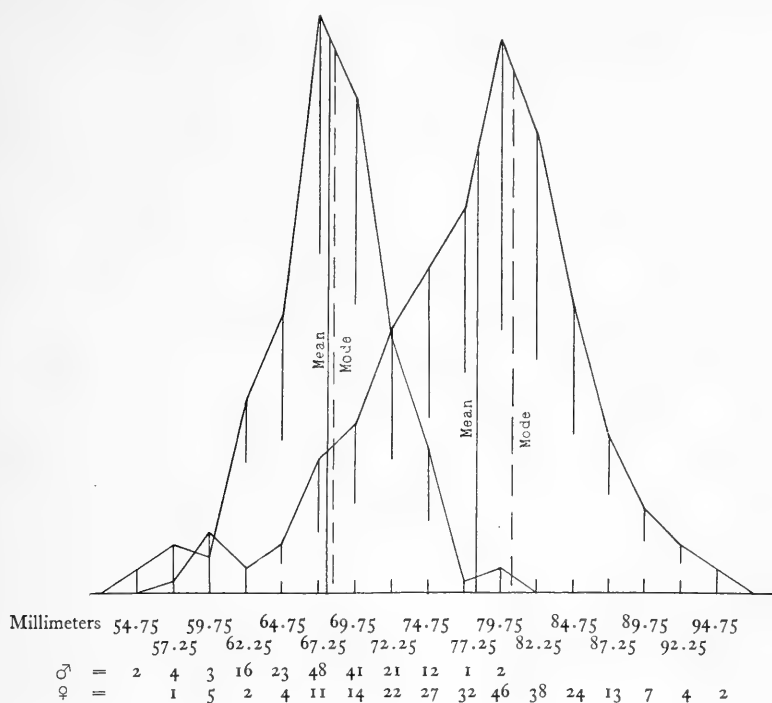


Fig. 6 Length of body.

### 5 Correlation

The degrees of correlation were determined for each character with every other, again treating the sexes separately. In all 144 correlation tables were drawn and indices calculated.<sup>3</sup> The results are given in Tables V to XVII. The averages derived from these tables are given in condensed form in Tables XVIII, XIX. (The probable errors of the coefficients were determined but are omitted from the tables.)

$$^3 r (\text{coefficient of correlation}) = \left( \frac{\sum (x' y')}{n} - v_1' v_1'' \right) \frac{1}{\sigma_1 \sigma_2}$$

TABLE V

*Indices of correlation between total weight and*

	♂	♀
Length total.....	.904	.924
Length body.....	.903	.925
Length thigh.....	.835	.893
Length shank.....	.697	.855
Length foot.....	.864	.878
Length leg.....	.871	.863
Length head.....	.838	.866
Width mouth.....	.746	.866
Weight gastrocnemii.....	.864	.915
Weight liver.....	.776	.808
Length alimentary canal.....	.376	.676
Weight ovaries.....		.838
Average all characters.....	.789	.859
Average external characters.....	.835	.887
Average internal characters.....	.576	.774

TABLE VI

*Indices of correlation between total length and*

	♂	♀
Weight total.....	.904	.924
Length body.....	.944	.946
Length thigh.....	.919	.977
Length shank.....	.903	.956
Length foot.....	.942	.947
Length leg.....	.962	.945
Length head.....	.863	.902
Width mouth.....	.830	.944
Weight gastrocnemii.....	.883	.947
Weight liver.....	.666	.738
Length alimentary canal.....	.487	.656
Weight ovaries.....		.699
Average all characters.....	.846	.882
Average external characters.....	.906	.943
Average internal characters.....	.577	.698

TABLE VII

*Indices of correlation between length of body and*

	♂	♀
Weight total.....	.903	.925
Length total.....	.944	.946
Length thigh.....	.832	.933
Length shank.....	.743	.863
Length foot.....	.863	.909
Length leg.....	.876	.946
Length head.....	.854	.881
Width mouth.....	.765	.893
Weight gastrocnemii.....	.856	.904
Weight liver.....	.709	.769
Length alimentary canal.....	.472	.638
Weight ovaries.....		.756
Average all characters.....	.802	.864
Average external characters.....	.848	.911
Average internal characters.....	.590	.721

TABLE VIII

*Indices of Correlation between length of thigh and*

	♂	♀
Weight total.....	.835	.893
Length total.....	.919	.977
Length body.....	.832	.933
Length shank.....	.857	.978
Length foot.....	.865	.963
Length leg.....	.940	1.000+
Length head.....	.774	.900
Width mouth.....	.775	.931
Weight gastrocnemii.....	.845	.923
Weight liver.....	.615	.731
Length alimentary canal.....	.499	.567
Weight ovaries.....		.646
Average all characters.....	.796	.870
Average external characters.....	.849	.944
Average internal characters.....	.557	.648

TABLE IX

*Indices of correlation between length of shank and*

	♂	♀
Weight total.....	.697	.855
Length total.....	.903	.956
Length body.....	.743	.863
Length thigh.....	.857	.978
Length foot.....	.755	.928
Length leg.....	.910	.944
Length head.....	.711	.908
Width mouth.....	.788	.881
Weight gastrocnemii.....	.866	.914
Weight liver.....	.539	.671
Length alimentary canal.....	.452	.582
Weight ovaries.....		.640
Average all characters.....	.747	.843
Average external characters.....	.803	.914
Average internal characters.....	.495	31.6

TABLE X

*Indices of correlation between length of foot and*

	♂	♀
Weight total.....	.864	.878
Length total.....	.942	.947
Length body.....	.863	.909
Length thigh.....	.865	.963
Length shank.....	.755	.928
Length leg.....	.954	.976
Length head.....	.713	.868
Width mouth.....	.764	.891
Weight gastrocnemii.....	.813	.888
Weight liver.....	.643	.750
Length alimentary canal.....	.366	.604
Weight ovaries.....		.631
Average all characters.....	.777	.853
Average external characters.....	.837	.916
Average internal characters.....	.505	.662

TABLE XI

*Indices of correlation between length of leg and*

	♂	♀
Weight total.....	.871	.863
Length total.....	.962	.945
Length body.....	.876	.946
Length thigh.....	.940	1.000+
Length shank.....	.910	.944
Length foot.....	.954	.976
Length head.....	.849	.852
Width mouth.....	.813	.913
Weight gastrocnemii.....	.874	.886
Weight liver.....	.638	.731
Length alimentary canal.....	.466	.554
Weight ovaries.....		.640
Average all characters.....	.832	.854
Average external characters.....	.894	.925
Average internal characters.....	.552	.642

TABLE XII

*Indices of correlation between length of head and*

	♂	♀
Weight total.....	.838	.866
Length total.....	.863	.944
Length body.....	.854	.881
Length thigh.....	.774	.900
Length shank.....	.711	.908
Length foot.....	.713	.868
Length leg.....	.849	.852
Width mouth.....	.747	.845
Weight gastrocnemii.....	.744	.894
Weight liver.....	.649	.647
Length alimentary canal.....	.325	.594
Weight ovaries.....		.647
Average all characters.....	.733	.820
Average external characters.....	.788	.884
Average internal characters.....	.487	.629



TABLE XIII

*Indices of correlation between width of mouth and*

	♂	♀
Weight total.....	.746	.866
Length total.....	.830	.902
Length body.....	.765	.893
Length thigh.....	.775	.931
Length shank.....	.788	.881
Length foot.....	.764	.891
Length leg.....	.813	.913
Length head.....	.747	.845
Weight gastrocnemii.....	.768	.873
Weight liver.....	.411	.695
Length alimentary canal.....	.379	.519
Weight ovaries.....		.591
Average all characters.....	.708	.817
Average external characters.....	.777	.888
Average internal characters.....	.395	.602

TABLE XIV

*Indices of correlation between weight of gastrocnemii and*

	♂	♀
Weight total.....	.864	.915
Length total.....	.883	.947
Length body.....	.856	.904
Length thigh.....	.845	.923
Length shank.....	.866	.914
Length foot.....	.813	.888
Length leg.....	.874	.886
Length head.....	.744	.894
Width mouth.....	.768	.873
Weight liver.....	.667	.726
Length alimentary canal.....	.508	.628
Weight ovaries.....		.700
Average all characters.....	.790	.850
Average external characters.....	.835	.905
Average internal characters.....	.588	.684

TABLE XV

*Indices of correlation between weight of liver and*

	♂	♀
Weight total.....	.776	.808
Length total.....	.666	.738
Length body.....	.709	.769
Length thigh.....	.615	.731
Length shank.....	.539	.671
Length foot.....	.643	.750
Length leg.....	.638	.731
Length head.....	.649	.647
Width mouth.....	.411	.695
Weight gastrocnemii.....	.667	.726
Length alimentary canal.....	.383	.618
Weight ovaries.....		.702
Average all characters.....	.609	.716
Average external characters.....	.631	.727
Average internal characters.....	.383	.660

TABLE XVI

*Indices of correlation between length of alimentary canal and*

	♂	♀
Weight total.....	.376	.676
Length total.....	.487	.656
Length body.....	.472	.638
Length thigh.....	.499	.567
Length shank.....	.452	.581
Length foot.....	.366	.604
Length leg.....	.466	.554
Length head.....	.325	.594
Width mouth.....	.379	.519
Weight gastrocnemii.....	.508	.628
Weight liver.....	.383	.618
Weight ovaries.....		.465
Average all characters.....	.428	.592
Average external characters.....	.433	.602
Average internal characters.....	.383	.542

TABLE XVII  
Indices of correlation between weight of ovaries and

	♀
Weight total.....	.838
Length total.....	.699
Length body.....	.756
Length thigh.....	.646
Length shank.....	.640
Length foot.....	.631
Length leg.....	.640
Length head.....	.647
Width mouth.....	.591
Weight gastrocnemii.....	.700
Weight liver.....	.702
Length alimentary canal.....	.465
Average all characters.....	.663
Average external characters.....	.679
Average internal characters.....	.584

TABLE XVIII  
Average correlation coefficients

	All characters		External characters		Internal characters	
	♂	♀	♂	♀	♂	♀
Weight total.....	.789	.859	.835	.887	.576	.774
Length total.....	.846	.882	.906	.943	.577	.698
Length body.....	.802	.864	.848	.911	.590	.721
Length thigh.....	.796	.870	.849	.944	.557	.648
Length shank.....	.747	.843	.803	.914	.495	.631
Length foot.....	.777	.853	.837	.916	.505	.662
Length leg.....	.832	.854	.894	.925	.552	.642
Length head.....	.733	.820	.788	.884	.487	.629
Width mouth.....	.708	.817	.777	.888	.395	.602
Weight gastrocnemii.....	.790	.850	.835	.905	.588	.684
Weight liver.....	.609	.716	.631	.727	.383	.660
Length alimentary canal.....	.428	.592	.433	.602	.383	.542
Weight ovaries.....		.663		.679		.584
Averages.....	.727	.799	.779	.852	.507	.652
Averages.....	.763		.8155		.5795	

TABLE XIX

*Average degrees of correlation*

	♂	♀
External characters with external characters only.....	.837	.912
External characters with all characters.....	.782	.851
All characters with all characters.....	.727	.799
External characters with internal characters only.....	.532	.669
Internal characters with all characters.....	.5185	.657
Internal characters with internal characters only....	.383	.595

A preliminary inspection of these tables shows that the degrees of correlation are relatively high throughout. Many of the coefficients exceed .9 and the lowest is .38. Again we find the two sexes entirely separate with respect to the degrees of correlation. The females show markedly higher degrees of correlation than the males, there being in 66 cases only three in which the coefficient is lower in the female. In two of these exceptions the observed difference is less than the probable error. These differences disappear in the averages of course so that considering each character with reference to all the others we may say that *the females are uniformly more perfectly correlated than the males*. The average coefficients for all characters are .727 — males and .799 — females; that is, the female coefficients are 10 per cent higher than those of the males. It will be recalled that the females were also about 23 per cent more variable than the males so that *the more variable sex is at the same time the more perfectly correlated: a high degree of variability being associated here with a high degree of correlation*.

Again we find that the characters measured fall into two quite distinct groups with respect to their coefficients of correlation much as with respect to their coefficients of variability. All of the external dimensions together with total weight and weight of gastrocnemii form a fairly uniform group and show a much higher degree of correlation than do the weights of liver and ovaries and length of alimentary canal, which form another distinct group. Table XIX summarizes these relations and shows the average coefficients of correlation of the "external" characters among themselves to be .837 — male, and .912 — female, while the "internal" characters among themselves give only .383 — male and .595 — female. The average coefficients of external characters with

all characters are .782 — male and .851 — female, and of internal characters with all characters .518 — male and .657 — female. That is, with respect to the organism as a whole, as measured by these thirteen characters, the external characters are in the male 51 per cent and in the female 29.5 per cent more perfectly correlated than the internal characters: while among themselves only the external characters are in the male 118.5 per cent and in the female 53 per cent more perfectly correlated than the internal characters. It will be recalled that the internal characters were found to be about four times as variable as the external characters so that we now find *the more variable characters to be the less perfectly correlated: a high degree of variability is associated here with a low degree of correlation.*

In any correlation table four groups of individuals are distinguished. Of these one group consists of individuals which are below the average of each of the characters with respect to which the table has been drawn, and another group of individuals above the average of each character. These are the two positive quadrants of the table. A correlation table therefore gives an opportunity for comparing in certain respects these two classes. The average deviation from the means, of each of these classes of individuals was determined<sup>4</sup> in the 144 possible pairs, the sexes as usual being treated separately. In 138 of the 144 possible opportunities for comparison the average deviation from the means was greater among those below the averages. For purposes of comparison the average deviations of the two groups of a single table were reduced to ratios of those above to those below the averages. The actual ratios varied between .7 and 2.3, the general average being 1.44. That is, the "scatter" about the regression line among those individuals which are below the average size is nearly one-half (44 per cent) greater than it is among those of more than average size.

Again the sexes represent distinct series for in 54 of the 66 possible cases where similar pairs of characters are under consideration

$$^4 \quad \frac{\sum (x_1' y_1')}{n_1} \quad \text{and} \quad \frac{\sum (x_2' y_2')}{n_2}$$

in the two sexes, the males show a lower ratio than the females. That is to say, in the males the "scatter" is more nearly the same in the larger and smaller groups than is the case in the females. In the males the average ratio is 1.309 in the females 1.562, the difference being about 7.5 times the probable difference so that there is no doubt that the sexes are actually distinct in this regard.

#### IV DISCUSSION OF THE DATA AND COMPARISONS WITH OTHER FORMS

##### *1 Numerical Ratio between the Sexes*

Questions relating to sex are receiving renewed attention and any data upon the subject should be welcome, especially any concerning the lower vertebrates where our information is quite deficient. A few facts may be mentioned here therefore regarding the numerical ratio between the sexes.

Among the mammals and birds the ratio between adult males and females usually approximates 1.0 but frequently shows an excess of males. Various authors have given data showing ratios of from 977 to 1158 males per 1000 females. Among the lower vertebrates however a very considerable inequality is usual with an excess of females. Among the bony fishes Fulton ('91) gives data for 26 species of food fishes. In only 14 species were more than 100 individuals observed, and of these, ten showed the males to be much less numerous than females, the ratios varying from 750 : 1000 (Cod—*Gadus*,  $n = 957$ ) down to 244 : 1000 (Gurnard—*Trigla*,  $n = 1299$ ). In two species—Ling (*Molva*) and Herring (*Clupea*), the ratio was practically 1.0 and in two others the males outnumbered the females—in the Flounder (*Pleuronectes*,  $n = 312$ ) 1622 : 1000 and the Lesser Sand Eel (*Ammodytes*,  $n = 858$ ) 1183 : 1000.

Among 100 examples of *Necturus* collected for examination, Bumpus ('97) found 37 males, a ratio of 587 males to 1000 females. In the frog (*Rana fusca*) Griesheim ('81) made several counts of tadpoles in which the sex was just determinable and found the number of males per 1000 females to vary between 353 and 375. Cuénot ('99) reports a ratio of 820 : 1000, males to females in the

grass-frog (*Rana temporaria*). The observed ratio here in the toad (*Bufo*) of 658 : 1000, males to females is therefore of the same order as that of other lower vertebrates. At first sight the smaller number of male toads, taken in connection with the fact that their variability is much less than that of the females might seem to indicate that their reduced number was the result of a selection more stringent among them than among the females. The data themselves however give no indication of this and that this is probably not the case is suggested by data from Pflüger ('81). He showed that the relative numbers of males and females in *Rana fusca* were not sensibly different in the natural population and among individuals resulting from artificial fertilization. His figures give for 806 young individuals a ratio of 357 : 1000, males to females, and for older forms 374 : 1000. His results therefore agree with those of Griesheim which his experiments were designed to check. Among adults he found the ratio 963 : 1000, indicating that the greater death-rate was among the females, but the number counted here was too few to give positive results.

In a very general way it seems true that there is a relation between the ratio of males to females and a condition of monogamy and polygamy. Among the higher forms where an approximation at any rate to monogamy is the general rule the sexes are about equal numerically, with a tendency toward excess of males. While among the lower forms where one male more usually may fertilize the ova of several females, especially if fertilization is external, there is a decided preponderance of females. There are frequent exceptions of course but the general relation seems to exist. Much more information is needed concerning the ratio between the sexes, especially in the lower vertebrates and also regarding their mating habits.

While there are no definite data which afford a satisfactory explanation of this sex disproportion, it may be worth while to suggest that it would result from a reproductive (genetic) selection of those individuals which were more largely female producing. The usual overproduction of spermatozoa together with the fact that the ova of several females are fertilized by a single male would give a decided advantage to species in which females pre-

ponderate and would afford a sufficient basis for the action of a reproductive selection in this direction. Among monogamous forms the excess of males would similarly be an advantageous condition, insuring the fertilization of every female and might similarly result from a reproductive selection in this direction.

One farther point should be mentioned in this connection though not strictly apropos. The collection of toads giving the ratio of 658 males to 1000 females involved, as we have seen, practically the entire population. Of the first half of the population collected *i. e.*, of the first 220 specimens, 108 were males and 112 females, a ratio of 964 : 1000. These were collected purely at random, being taken as they were found and no rejections whatever made. The first 220 individuals represented what might with perfect right be called a "random sample" and yet collection of the entire population showed how far this was from being a true sample of the whole. The discrepancy was utterly beyond detection by any statistical method and yet the usual precautions in the collection of the material were fully observed. In the second half collected the ratio of males to females fell to 438 : 1000.

A fairly close study of the habits of the toad during the entire summer had not disclosed the fact that a true sample was not being obtained but the complete data show that this was the case. The reason for this can not now be determined certainly. It might have been the result of such a fact as that the males appeared earlier in the evening than the females so that collections made early in the season when material was abundant and enough for the next day's measurement could be obtained in a few minutes would consist of a larger proportion of males than later in the season when the search for material was necessarily continued frequently until midnight. Whether this was the case or not can not now be said. In itself the point is of no particular importance but it serves to emphasize the thoroughness with which the preliminary study of an organism and its habits must be made when samples of a population are to be used as representative of the whole. Furthermore it affords a clear illustration of a fact occasionally overlooked in the collection of data, namely that the statistical method gives no exact indication as to the truly representa-



tive character of a sample. The determination of the "probable error" of a datum can not take into account any error of observation.

## 2 Variability

### a Comparative Variability of the Sexes

The relative variability of man and woman is a much discussed subject and exact data are fairly abundant but among other forms exact data are by no means abundant. In man while neither sex is uniformly the more variable it seems now that, quite contrary to the former belief, the female is usually more variable than the male. Pearl ('05) gives a summary of the coefficients of variability in man compiled from various sources. Of 41 characters of many kinds the female is more variable in 25, the male in 16, the difference rarely exceeding 15 per cent of the male value. Pearson ('97) also showed that on the whole the ratio between the averages of a large number of coefficients of variability in various races in man and woman is .973, showing that is, a slightly greater variability in woman. Among other mammals Davenport and Bullard ('96) show the variability in the number of Müllerian glands in swine to be  $2\frac{1}{2}$  per cent greater in the male than in the female. Minot's ('91) data on the weight at birth of male and female guinea pigs (*Cavia*) give coefficients of 23.94 and 22.62, respectively. Montgomery ('96) states that in a number of species of birds the males are more variable than the females in about 60 per cent of the measurements made; his results can not be stated in terms of the coefficient of variability. In the shrike (*Lanius*) Strong ('01) measured four external characters and found very slight differences between the sexes, the male perhaps being slightly the more variable.

In *Necturus* Bumpus ('97) found the males less variable than the females with respect to abnormally placed pelves, but again the results can not be expressed as coefficients. In the toad (*Bufo*) we have found not only that the females are more variable than the males in every one of the twelve characters compared but that they are very decidedly so—from 6.6 per cent to 38.4 per cent, so

that we have here a form quite unique among vertebrates hitherto reported, in both the extent and uniformity of the difference in variability of the sexes. The matter will be discussed in connection with the subject of correlation.

#### b The Frequency Polygons

We have noted that distributions of all the characters in both sexes are skew and that the skewness in nearly all cases is negative, being positive only in the liver and gastrocnemii of male. The negative skewness, which means merely that the majority of individuals are below the modal value of a type, was to have been expected because of the inclusion of individuals of all ages of one year or more. It is probably merely an indication that the younger outnumber the older in our material, at any rate we need look no farther than this for a sufficient explanation of the skewness. It is indeed surprising that the skewness is so slight indicating that in this general population survival to middle or old age is usual. The positive skewness of the livers is an expression of the fact that in these as in most vertebrates the liver is relatively larger in the younger animals.

#### c Comparative Variability of External and Internal Characters

There have been remarkably few exact studies of the variability of internal (visceral, etc.) characters and as far as I have yet been able to discover these have been observed only in man with but a single exception. Greenwood ('04) has given the coefficients of variability for the heart, liver, spleen and kidney in a hospital population of males. Pearson ('97) has calculated the coefficients for the heart, liver, kidney and brain from the data published by Clendinning, Reid and Peacock, and Sims published in 1838, 1843, 1846, 1854: probably these data have not the accuracy required by present statistical methods, indeed it is known that in securing them many were rejected on account of their supposed "abnormality." The coefficients of these human viscera vary between 38.2 and 14.3. The brain of course has furnished a more complete series of data. These have recently been collected and summa-

rized by Pearl ('05): it will be noted that these coefficients are considerably lower than those of the older series by the authors just mentioned, and should be used in place of them. The coefficients of variability of all of these organs are given in Table XX.

TABLE XX  
*Coefficients of variability of internal characters*

Human—	♂	♀
Weight of spleen (Greenwood).....	38.21	
Weight of heart (Greenwood).....	17.42	
Weight of kidney (Greenwood).....	16.80	
Weight of liver (Greenwood).....	14.80	
Weight of heart (Reid and Peacock [Pearson]).....	19.82	20.70
Weight of liver (Reid and Peacock [Pearson]).....	14.32	22.23
Weight of kidney (Reid and Peacock [Pearson]).....	20.49	22.53
Capacity of lungs (breathing capacity) (Galton [Pearson])....	16.6	20.4
Weight of brain (Reid and Peacock, Clendinning, Sims, Bischoff, Pearson).....	8.07 to 10.25	7.93 to 10.64
Weight of brain (Pearl).....	7.59 to 8.85	7.09 to 8.72
Swine—		
Number of Müllerian glands (Davenport and Bullard).....	48.0	
Toad—		
Weight of gastrocnemii.....	24.50	28.94
Weight of liver.....	33.34	35.55
Weight of ovaries.....		53.23
Length of alimentary canal.....	9.81	12.34

The great mass of data on variability is drawn from measurements of body-weight, stature, skull and other skeletal characters of similar nature. Of these, excluding body-weight as a composite of both external and internal characters, the coefficients range from 5.57 to 3.15, an average of 48 different characters in both sexes giving 4.2. In other vertebrates we have essentially similar magnitudes ranging from 8.80 to 2.69. (Lönnerberg ('93) gives body-lengths of 141 specimens of *Petromyzon fluviatilis* which upon calculation show a coefficient of 9.66.)

Obviously there is an enormous difference in the variability of these external and internal<sup>5</sup> characters. In the toad we have

<sup>5</sup> The use of the convenient words "external" and "internal" to distinguish these two classes of characters is perhaps justifiable though not exact. As external we may include characters such as stature, length of limbs or limb bones, number of vertebrae, indices and other skull measurements except perhaps capacity, the position, number or size of external parts, etc.; in general all such characters as func-

seen that the external characters are about four times as variable as the internal characters, a relation similar in general to that in man where the internal characters are roughly four to five times the more variable. Brain characters hold an intermediate position being roughly only about twice as variable as external characters.

It is also significant that the variability of pure functions, as far as they have been measured, is of the same general magnitude as the variability of these internal organs. Table XXI summarizes data collected by Pearson ('97) from various sources.

TABLE XXI  
*Coefficients of variability of functional characteristics in man*

	♂	♀
Squeeze of hand (Porter).....	29.30 to 37.89	32.41 to 45.58
Squeeze of hand (Galton).....	13.4	21.4
Squeeze of hand (Cambd. Anthrop. Com.).....	13.64 (r)	18.42 (r)
	14.55 (l)	18.78 (l)
Strength of pull (Cambd. Anthrop. Com.).....	15.58	16.72
Strength of pull (Galton).....	15.0	19.3
Strength of pull (Quetelet).....	15.32	22.62
Keeness of eyesight (Galton).....	28.68	32.21
Keeness of eyesight (Cambd. Anthrop. Com.).....	33.25	32.93 (l)
		34.73 (r)
Dermal sensitivity (Galton).....	35.70	45.75
Swiftmess of blow (Galton).....	19.4	17.1

As an explanation of this difference in variability between internal and external characters Pearl ('05) has suggested tentatively that the greater variability of the internal characters is due partly to the fact that they depend to a very considerable degree for their value upon the general metabolic condition of the organism as a whole at the time of measurement, and partly to the fact that in visceral characters the thing measured is not the thing with which natural selection, as far as it has acted at all, has had to do. With respect to the first suggestion it might be objected that while this

tion "passively," *i. e.*, whose value to the organism lies chiefly in position, or in a numerical or dimensional relation. As internal we should include organs whose function is of a more "active" sort—muscles, nerve-centers, glandular organs of all kinds, etc., structures whose value to the organism lies in a metabolic rather than a mechanical relation. The distinction is not precise, certain organs may possess values of both kinds, and yet the distinction is broad enough to be useful.

may be true for such characters as the weight of the spleen or liver it would be less true for such as the weight of the heart or brain or length of the alimentary canal. And farther, the values of many external characters are easily subject to modification by the general metabolic condition of the organism, particularly during the early period of growth (*e.g.*, Vernon ('95)) and that many of these modifications may be overcome by compensatory growth should conditions of life change. The second suggestion carries more force. It amounts practically to saying that at present we have no means of measuring the actual value of these internal characters which approaches accuracy. A measurement of the mass of the liver, for example, gives no exact information as to its functional worth. There is no legitimate reason for supposing that there is an exact ratio between the size of a viscus and its functional value, indeed the ratio between mass and efficiency may be inverse and the better the tissue is functioning the smaller need its mass be to carry on its work in the life of the organism.

In the statistical study of the characteristics of animals it should be borne in mind constantly that it is the functional value of a character to its possessor which is the bearing point of natural or any other form of selection. It makes no difference to a toad how long his legs may be but only how far or how fast he can jump. If the length of the legs or their segments is an exact indication of their ability to function, then only are we justified in using their lengths as data of actual evolutionary significance. Similarly, to the individual toad the weight alone of the liver is a matter of no consequence, only its ability to secrete and metabolize in both qualitative and quantitative relations, and unless we can demonstrate a close relation between bulk and efficiency, again the data themselves do not afford material for study of the evolutionary significance of its variability in weight.

That there is a very close relation between the dimensions of external characters and their functional value is probably true but the fact of their low degree of variability is not sufficient alone to prove it. The segments of the legs, for example, form a system of levers whose action depends largely upon their relative lengths and proportions. Their value lies almost wholly in their purely mechan-

ical or dimensional relations. This is indicated by the fact that the ratios between the average values of external characters are the same among individuals of various sizes. Table III showed the exact correspondance between these ratios in the male and female toad, and Donaldson ('98) has thoroughly demonstrated the same fact in a series of bullfrogs of various sizes. In frogs of all sizes and both sexes the sum of the leg bones is a nearly constant fraction of the length of the entire frog, and the proportional lengths of the several bones are also nearly constant. So it is for most external characters.

But with internal characters there is good reason for questioning the exactness of this relation between size and efficiency. As a matter of fact there are almost no data bearing upon the subject from which to form an opinion. It is generally believed from the evidence so far produced that there is no exact relation between size of brain and intellectual ability. There is a fairly close correlation between size of head and size of brain but Pearson (02a) was unable to find any appreciable correlation between size of head and intellectual ability. The only data which I have been able to find comparing the value of a function with physical measurement of the organ functioning is in the case of muscle. Weber's law that the absolute power of a muscle is proportional to its cross-sectional area (*i. e.*, to the number of fibers) is true only in the most general way. Weber found the absolute power of the human gastrocnemius and soleus to be about 1 kg. per sq. cm., while others have found values of 6.25 and 8.0 kg. in other human muscles. In the frog's muscle Weber gives 0.6 kg. per sq. cm. as the absolute power while Rosenthal ('67) gives 2.8 to 3.0 kg. for frog's semimembranosus and adductor magnus and 1.0 to 1.2 kg. for the gastrocnemius. Howell ('05) summarizes the matter by stating that "the absolute power of a frog's muscle of 1 sq. cm. cross-area is estimated at from 0.7 to 3.0 kgs."—rather wide limits. Indeed when we consider the number and complexity of both external and internal factors affecting such a comparatively simple process as muscular contraction it seems useless to attempt at present any exact comparison between size and efficiency. Many of the phenomena of

muscular contraction show that the efficiency of a muscle depends after all fully as much upon the activity of the nerve centers controlling it as upon the characteristics of the muscle itself. The coefficients of variability of strength of pull, squeeze of hand, etc., are measures of the variability of the central nervous system as much as of the muscles involved in the action. And when we come to consider the action of absorbing surfaces or secreting organs it is simply impossible with our present knowledge and technique to make any more than the most general statements about the relation between the size of such organs and their functional value or efficiency.

One farther consideration suggested above bears directly upon this matter. It is well known that the action of the central nervous system determines to a very considerable extent both the quantitative and qualitative results of the action of metabolizing organs. The functional activity of the digestive glands for example, is thus constantly modified without there being any detectible physical alteration (Pawlow and others), both the nature and amount of their secretions depending upon the action of the nervous system. The accurate adjustments of variations in the activity of these organs depend not upon the physical characters of the glands but upon the modifying influence of the nervous system in producing slight modifications of their internal metabolic processes. This of course is a factor entirely lacking when the efficiency of an organ is directly dependent upon its relatively fixed dimensions.

We must conclude therefore that measurements of the mass and dimensions of internal organs give data which can be used in an exact study only of these organs themselves without reference to their functional value to the organism as a whole: that they do not furnish evidence as to the precise efficiency of the organs, nor as to the effects of natural or other form of selection upon them in their functional relation; in this respect such data have only a general, not a precise, significance.

The extremely high degree of variability of visceral characters may then, it seems to me, be also in some part the result of a fact that lies at the basis of many of the practices of modern surgery,

namely, that such organs rarely function to the limit of their capacity. It is well known that large portions of many of the internal organs may be removed without causing any serious or sometimes even any visible disturbance of the physiology of the organism. The entire spleen, nearly the entire thyroid, or ovary, one entire kidney, and even large parts of the brain may thus be removed without visible effect. This it seems can only mean that ordinarily such organs are functioning only in small part, that they are working with a large margin of reserve; that their functional value is not determined by their size.

### 3 Correlation

#### a Comparative Degrees of Correlation in the Sexes

In the discussion of this subject we are again limited practically to human data. The relation here is quite similar to that of variability, *i. e.*, there is no uniform difference between the sexes but in general the female is perhaps slightly more perfectly correlated than the male. Of 29 pairs of coefficients among individuals of the same societies collected from various sources the females show higher correlation coefficients in but 14 and the average degree in the female is .442 as compared with .439 in the male—probably a non-significant difference. In swine (Davenport and Bullard '96) the coefficients between the numbers of Müllerian glands on the right and left sides of the body are .783 — female and .772 — male, a barely significant difference. Among the fishes the difference is somewhat more marked. Duncker (Vernon '03) states that in 40 pairs of coefficients 17 were unaffected by sex while in 11 the males, and in 6 the females were the more highly correlated. In the toad however, in correlation as in variability, there is both a decided and a uniform difference between the sexes. In 63 of the 66 pairs of coefficients (in two of the three exceptions the difference is less than the probable error) the female coefficients are higher than those of the male, the average degrees being .779 and .727, respectively.

We see then that in the toad the females are about 10 per cent better correlated than the males. Here then we have a relation



that may underlie the fact that in the same community where conditions of life are remarkably uniform, the females should be so much (23 per cent) more variable than the males. May it not be because the females are at the same time more perfectly correlated? Variations from the type of a given character are not disadvantageous because they are backed up by corresponding variations in other characters; the "balance of fitness" is maintained in the more variable individuals. The females remain more variable—are not selected down to the same level of variability as the males—simply because their variability involves not single parts or organs but groups of organs, in fact the entire organization. In other words, the organization of the females, abmodal as well as modal is more nearly a unit, the elements are better organized, less independent of one another, *i. e.*, simply "better correlated."

Schuster ('03) found a relation between sex and correlation in his crab measurements (*Eupagurus*) but there it was the males which were the more variable and more highly correlated. There seems therefore frequently to be a relation between sex and correlation as well as between sex and variability. Whether the relation is actually between variability and correlation will be discussed presently.

#### b Comparative Degrees of Correlation in External and Internal Characters

In the toad the average degrees of correlation in both sexes of the external and internal characters are .815 and .579, respectively (Table XVIII), and it should be borne in mind that it is the internal characters which are the more variable. In man, to which we are limited for comparative data among vertebrates, the relation is similar, as shown in Table XXII compiled from a number of sources. And here too the internal characters are the more variable. The coefficients of the brain are similar in general to those of the viscera ranging between .17 and .40 while the skeletal characters, excluding the skull, show an average correlation of .70: the skull resembles the brain in this respect.

In general these coefficients are comparatively high in the toad, both in external and internal characters: I know of no form in

which the coefficients are uniformly as high as here. The relation between the correlation of external and internal characters bears out the general conclusions reached in the discussion regarding their variability. The relative correlation of the brain in man has been mentioned by Pearl ('05), who points out that its correlation coefficients are of the same general magnitude as those of visceral characters. It should be noted however that the skull coefficients are of this same magnitude, quite unlike the other

TABLE XXII

*Coefficients of correlation—internal and external characters*

Human—	
Heart and liver.....	.278
Heart and spleen.....	.265
Heart and kidney.....	.400
Brain and various skeletal characters, excluding skull.....	.17 to .35
	average = .21
Brain and various skull characters.....	.36 to .55
	average = .47
Various skeletal characters, excluding skull, average.....	.70
Various skull characters, average.....	.35
Toad—	
Gastrocnemii and liver.....	.696
Gastrocnemii and alimentary canal.....	.568
Gastrocnemii and ovaries.....	.700
Liver and alimentary canal.....	.500
Liver and ovaries.....	.702
Alimentary canal and ovaries.....	.465
Various external characters, average.....	.875
Human—	
Strength of pull and height (Pearson '99).....	.216 to .303
Strength of pull and weight (Pearson '99).....	.338 to .545
Mental ability and various physiological characters (Pearson '06).....	.06 to .45
Mental ability and various structural characters (Pearson '06).....	.00 to .11

skeletal characters. This indicates that the characteristics of the skull are more dependent upon the brain characters than vice versa.

The relation between variability and correlation as indicated by these and other data may be mentioned here. We have seen that in the toad conditions of high variability and high correlation are associated in the female sex. The same relation has been noted in the male of *Eupagurus* by Schuster ('03), and among

plants a similar relation has been found between variability and correlation. (See *e.g.*, Weldon's ('01) recalculation of MacLeod's data in *Ficaria*.) On the other hand, we have seen also that in all the data available in internal characters the higher variability is associated with the lower correlation. As a specific instance may be mentioned some of the figures given by Greenwood ('04). In her study of the human viscera she found that the normal condition is one of low variability and high correlation as contrasted with a relation of high variability and low correlation among those diseased or in "general poor health." Pearson ('02b) in one of his *Mathematical Contributions to the study of evolution* demonstrates by a purely mathematical method that natural selection must determine the amount of correlation, that indeed, it is probably the chief factor in the production of correlation throughout the constitution of the organism even though it may operate occasionally upon single characters. This certainly agrees with the biologically determined facts. He farther establishes mathematically the general principle that "intensity of selection connotes a lessening of correlation," that a condition of lower variability must be associated with lower correlation. For example supposing the correlation between the lengths of tibia and femur to be .70, if selection reduces the variability of the tibia by 50 per cent the correlation will then be but .44—a reduction of 37 per cent. The correlation between organs related only indirectly would be similarly reduced though not to quite the same extent.

Pearson does not make it sufficiently plain that this relation would hold only for the same pairs of characters before and after a period of selection. It is evident that, speaking in general terms, a condition of higher variability is frequently associated with a higher correlation, and that also a lower variability is associated with a lower correlation. But that this is not a necessary relation is clear when we compare the internal and external characters on this basis. That the relation mentioned by Pearson is not a necessary one is again evidenced by Greenwood's data. The following table summarizing some of these data shows that the variability of the weights of the viscera is higher but the correlation at the same time lower in a general hospital population than in a popula-

tion classed as "healthy," *i. e.*, the relation between variability and correlation is inverse.

	General Hospital Population	"Healthy Postmortems"
Coefficient of variability in	(males)	(males)
Weight of heart.....	32.39	17.71
Weight of liver.....	21.12	14.80
Weight of spleen.....	50.58	38.21
Weight of kidney.....	24.62	16.80
Coefficient of correlation between		
Heart and liver.....	.1931	.2780
Heart and spleen.....	.1827	.2654
Heart and kidney.....	.2577	.4004

And again, with increase in age the variability of the heart and spleen was found to remain constant but the correlation coefficient increases steadily from .08 (25 to 35 years) to .25 (45 to 55 years). How this relation between variation and correlation can be explained mathematically is not clear but doubtless a mathematical "description" will be forthcoming once the biological fact is established.

The last point to be mentioned is the relation of the groups of larger and smaller individuals, *i. e.*, those above and below the average, to the regression line. We have seen that among those above the average in any respect the "scatter" about the regression line is markedly less than among those below the average. The full significance of this relation needs farther investigation but I take it that this is an indication that the larger, and therefore in general the older, individuals gradually approach more closely the *type as measured by the coefficients of correlation*. From the character of the distributions found in this material this would seem to be the result of growth rather than of selection upon the basis of variability. That the coefficients of correlation as well as of variability change with age is well known. That is to say in growth, organs behave as more or less independent units. It seems likely that normal processes of growth may consist to an important extent in the gradual approach toward a condition of more perfect general correlation. Data bearing upon this important point are still meager; it is unfortunate that the age of these

toads was not determinable with sufficient accuracy to afford evidence.

In conclusion, it seems that the general subject of correlation is one of increasing importance. Attention has been so largely given to the subject of variation that the facts of correlation, which may prove to be the more significant, have not received their due share of attention.

The "balance of fitness" which is the result of growth or selection is not a condition merely to be guessed at or estimated; it is measurable by a series of correlation coefficients among a considerable number of characters. The "summum bonum" is a condition of high correlation, be the associated variability higher or lower. To be fit an organism must exhibit throughout its organization a certain degree of proportion of its parts. This condition of fitness is measurable by a series of correlation coefficients not only of external dimensions and skeletal characters but also of size and strength of muscles, the position of their insertions upon the skeletal elements, the size (though only an approximate indication of efficiency) of viscera and especially of nerve centers, and, were the technique possible, also of the efficiency of sense organs and of the accuracy and intensity of reactions. And lastly, looking in this direction, studies in correlation should include not only the relation of pairs of organs in a large number of individuals but farther of the relation between a large number of representative characters among single individuals. The collection of data upon this last topic is now in progress.

The Woman's College  
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